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БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

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PHYSIOLOGY

COMPARISON OF THE ELECTRICAL RESPONSE OF THE RETICULAR FORMATION, THALAMUS, AND CORTEX TO A SERIES OF IDENTICAL STIMULI FROM AN INDUCTION COIL

M. M. Bantsekina

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In recent years, many workers have shown that afferent impulses may reach the cortex by two routes, passing along either the classical lemniscal or along the nonspecific pathways. The nonspecific afferent system of the brain stem has its own nuclei and its own distinct tracts and comprises the reticular formation, localized in the brainstem (diencephalon, mesencephalon) and in some of the thalamic nuclei. This system has a widespread nonspecific projection onto the cortex.

It has been suggested [2, 7, 9] that the reticular formation does not carry out any specific function, but establishes an appropriate level of exitability which enables processes to take place which are induced by afferent impulses passing along the specific pathways.

It is also known [7,10] that all sensory impulses pass along many collaterals in the region of the reticular formation of the brainstem where they interact extensively by multisynaptic connections and lose their original sensory specificity.

Direct stimulation of the reticular formation of the brainstem induces the so-called "waking" reaction on the electroencephalogram (EEG), and the animal passes from a state of sleep to one of wakefulness. When this region is inactivated, either functionally or anatomically , the normal animal goes into coma and fails to react to any stimulus.

Work by foreign authors on the pathways for nociceptive stimuli has consisted of direct stimulation or of elimination of different parts of the brain stem [4,5,6,10].

V. G. Agafonov [1] showed that the flow of pain impulses (induced by placing the rabbit's foot in hot water) could be blocked by an injection of aminasin. It was natural to suppose that pain is developed in the higher nervous centers only after some more specific "nucleus" has been established at a lower level in the brainstem. Probably, such a physiological integration making the whole reaction specific takes place at the level of the reticular formation and in the hypothalamus [2].

The present investigation reports the development of electrical activity in the reticular formation of the brainstem, thalamus, and cortex in response to painful stimulation.

METHOD

The experiments were carried out on 40 unanesthetized rabbits weighing about 2 kg.

Three to four days before the experiment, the skull was trepined under local anesthetic, and electrodes were inserted in the following subcortical structures of the left side: 1) in the reticular formation of the brainstem (medial portion), 2) in the lemniscal system of the brainstem (lateral portion), 3) in the medial thalamic nucleus.

The placement of the electrodes in these structures was made using the coordinate system of Gangloff and Monnier [3].

The potentials from the cortex were led off from the sensory motor and temporal regions of the cortex, the electrodes being placed on the internal surface of the calvarium.

The bipolar buried electrodes were made from nichrome wire 100 μ in diameter, and the cortical electrodes from 500 μ diameter steel wire.

All the electrodes were fixed to the skull with phosphate cement. During the experiment, the animal was held in a special support ("hammock").

The skin of the hindfoot was stimulated with a series of identical stimuli from an induction coil.

Potentials from the brain were taken to an amplifier and recorded on a four-channel ink writer which also recorded the stimuli and time intervals.

At the start of the experiment, the strength of the current required to effect an alteration of the electrical activity in all the structures from which recordings were taken was determined. As the experiment proceeded, the stimulus strength was changed as circumstances dictated.

To avoid a conditioned reflex being developed and associated with the time of stimulation, the stimulus was applied at different time intervals from 30 seconds to 20 minutes.

After the experiment, a histological check on the position of the electrodes was made.

RESULTS

At the beginning of the experiment, the EEG of the brain structures studied gave a picture which was typical of the orienting and searching reaction: there was a desynchronization in the sensory motor cortex, and low amplitude synchronization in the reticular formation of the brainstem and thalamus.

After a certain time interval, during which the animal lay quietly in the support, the electrical potential changed and gradually went over to those characteristic of the resting condition. In the reticular formation of the brainstem and in the medial thalamic nucleus, the EEG "resting" rhythm consists of low amplitude waves at a frequency of 5-8 per second alternating with periods of various duration at different amplitudes. In the sensory motor region of the cortex, the "resting" rhythm of the EEG consists of waves at a frequency of 2-3 per second of varyng amplitude (usually high), and these appear as though distorted by rapid oscillations, at 25-30 per second and by periodically occurring bursts of "spindles". In the temporal cortical region, the "resting" rhythm consists of high amplitude oscillations, at a frequency of 5-6 per second.

For each animal, the period of "resting" occurred after a different interval of time; only after the appearance of the "resting" background of the EEG did we apply the series of identical induction shocks.

The reaction to the equal pain unconditioned stimulus consists of definitive and characteristic changes in the EEG: first the irregular rhythm of electrical activity of the reticular formation changes over to a very regular rhythm, at a frequency of 4-6 per second, i.e., it becomes synchronized, and secondly, in the sensory motor cortex, the initial "resting" current changes over to a high frequency, low amplitude oscillation, i.e., it becomes desynchronized; thirdly, in the temporal region of the cortex, there is a change toward a regular synchronized rhythm with potential variations at a frequency of 4-6 per second (Fig. 1, a).

It must be emphasized that in the structures studied, the alteration in the EEG develops in response to the application of identical and very short-lasting stimuli (lasting only a fraction of a second), while the change

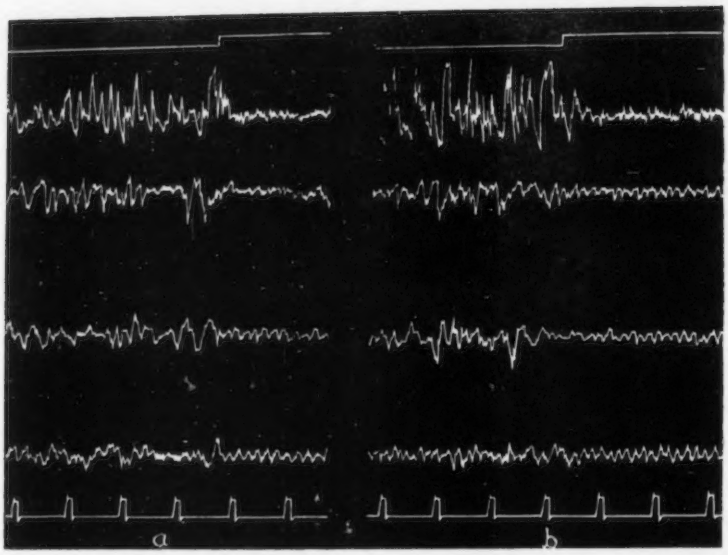

Fig. 1. Changes in the rabbit EEG in response to a series of identical electrical cutaneous stimuli. Curves, from above downwards: stimulus marker; sensory motor region of cortex; temporal region of cortex; medial division of the thalamus; reticular formation of the brainstem (superior corpora quadrigemina); time marker (1 second). a) Desynchronization in the sensory motor region of the cortex and steady rhythm in the reticular formation of the brain stem, thalamus, and temporal cortex, in response to a series of equal electrical cutaneous stimuli corresponding to 13 cm on the scale of the induction coil (5th stimulation); b) phenomenon of "bahnung" with repetitive application of the stimulus (strength of current 22 cm on induction coil) (reduced voltage produces an identical change in EEG).

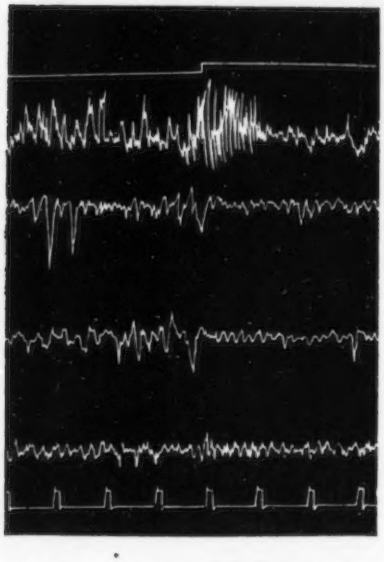

Fig. 2. Changes in the EEG of the reticular formation of the brainstem and thalamus, occur earlier than those in the sensory motor cortex, in response to application of the first stimulus. Traces as in Fig. 1.

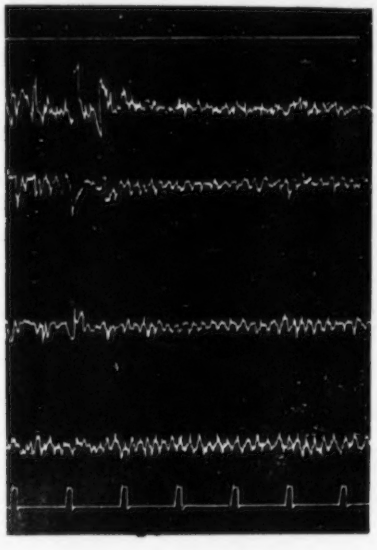

Fig. 3. Conditioned reflex changes in the electrical activity to any kind of sound (at end of experiment). Traces as in Fig. 1.

produced lasts form many seconds. The regular electrical oscillations in the temporal region are synchronous with those in the subcortical structures (reticular formation of the brainstem and medial thalamic nucleus).

Subsequent application of the same stimulus brings about the phenomenon of "bahnung", i.e., a smaller voltage will now produce an identical change in the electrical activity (Fig. 1,a,b).

It is particularly interesting to observe that the use of a weak electrical stimulus of a definite strength, brings about a dissociation in the time of the appearance of the EEG in the subcortical structures, as compared with the cortex; first of all, the electrical activity changes in the reticular formation and in the medial thalamic nucleus, but only after a period of 0.2-0.4 seconds does the same change occur in the sensory motor cortex (Fig.2), the EEG reaction of the subcortical structures occurs before that in the cortex.

A detailed analysis of this dissociation, shows that a gradual increases in the strength of the stimulus may have the effect that a single shock causes a typical EEG reaction which is shorter than usual in the brainstem and thalamus, while in the sensory motor cortical area, the same stimulation produces no visible changes in electrical activity. With subsequent repititive sitimulation at the same intensity, or with a stronger single stimulus (and the same recording rate), it is not possible to reveal any difference in the time of the appearance of the reaction at the different levels; the stronger the current and the higher the stimulus frequency, the more definite and prolonged is the complex reaction.

Thus, a single stimulation applied to the skin produces a fairly constant EEG reaction, which takes the form of a change in the rhythm of the reticular formation and of the medial thalamus, and which slightly precedes the desynchronization in the region of the sensory motor cortex; the reaction continues for as long as several seconds after the action of the single stimulus. By the end of the experiment, this EEG reaction has assumed the nature of a conditioned reflex, and the characteristic change in the rhythms of the reticular formation of the brainstem and the thalamus, as well as in the sensory motor and temporal cortical regions, develops in response to the slightest sound (Fig. 3).

Bearing in mind that stimulating the skin with a single shock produces, first, a change in the electrical activity of the reticular formation of the brainstem followed immediately afterwards by a change in the sensory motor cortex, it must be supposed that the complete pain reaction develops first at the reticular level, and that the excitation does not spread to the highest levels of the brain until later, as is revealed by the desynchronization of the potentials in the sensory motor cortex.

However, on account of the more rapid spread of the excitation along the lemniscal system [8], it is probable that the conditioned reflex pain reaction is due to impulses flowing back from the cortex along the corticofugal pathways to the reticular formation.

SUMMARY

Single cutaneous electrical stimuli evoke the following changes in cerebral electrical activity: synchronization in the reticular formation, medial thalamus, and temporal cortex, with desynchronization in the sensory motor cortex. Low voltage stimuli produce changes in the EEG of the reticular formation which appear earlier than do those of the sensory motor cortex. In the author's opinion, pain is first signaled at the level of the brainstem, and later the excitation moves up to the higher levels.

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DEVELOPMENT OF A STABLE EXCITATORY FOCUS IN THE SPINAL CORD OF WARM-BLOODED ANIMALS

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The mechanism of the formation of stable excitatory foci in the central nervous system is of great importance in describing nervous activity under normal and pathological conditions.

The idea of the dominant, which was put forward by A. A. Ukhtomskii who was influenced by the ideas of N.E. Vvedenskii, has been the subject of much further development by many workers [1-14].

However, until now, many of the questions associated with the conditions and mechanisms of the development of dominant zones in the central nervous system have not been sufficiently studied.

Thus, in the formation of the dominant, not enough is known about the coordinating function of the spinal centers. It is particularly important to study these centers in warm-blooded animals.

The present work deals with the detailed method of formation of a dominant in the spinal cord of the cat, and the prolonged influence of subthreshold, threshold, and suprathreshold stimulation of an afferent nerve.

METHOD

The experiments were carried out on decerebrate cats. Dissections were made of the semitendinosus muscles and peroneal nerves in both hindlimbs, as well as of the ulnar nerves in both front legs.

Stimulation of the central ends of the nerves was effected with an electric current from an induction coil, or from a stimulator working at a frequency of 50 cps. The reflex contractions of the muscles of the antagonists (semitendinosus muscles) were recorded.

The dominant was developed in the flexor center of the hindlimbs by application of subthreshold, threshold, or suprathreshold stimulation of long duration to the peroneal nerve.

Other afferent nerves, including the peroneal nerve of the opposite side and the ulnar nerves of both sides, were stimulated to supply supplementary stimulation for the purpose of revealing the dominant.

At first, the peroneal nerve of one side was stimulated, and the thresholds for the reflex contraction of the semitendinosus muscles of the ipsi- and contralateral sides were determined; after this, the intensity of the stimulation was varied, and the reflex contractions of the same muscles were recorded.

In other tests, the peroneal nerve of one side was stimulated, causing reflex contraction of the semitendinosus of the ipsilateral side, and in addition, the peroneal nerve of the contralcteral side and the ulnar nerves of both sides were also stimulated. In this case, stimulation of the peroneal nerve of the contralateral side normally

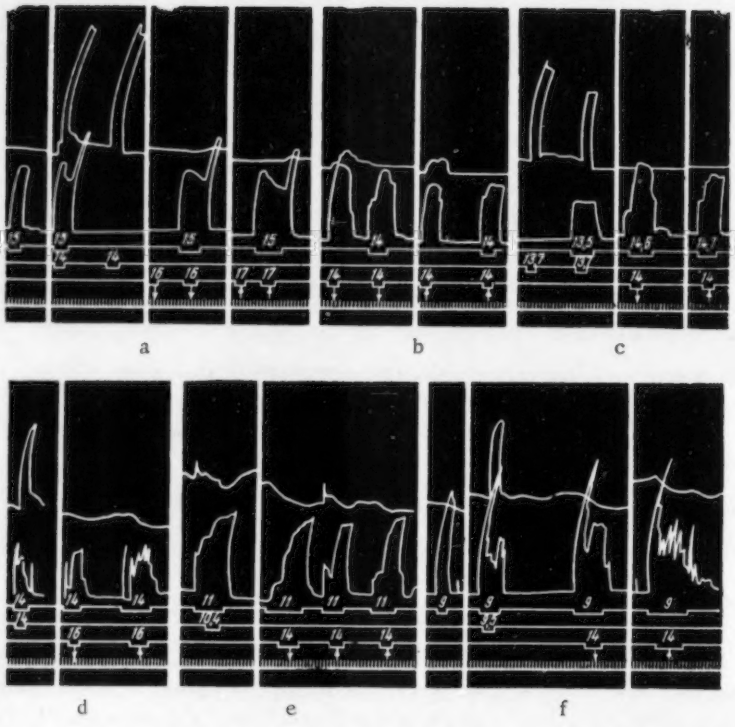

Fig. 1 Development of a dominant zone in the flexor center of the spinal cord of the cat in response to prolonged subthreshold stimulation of the ipsilateral peroneal nerve. a) Initial condition; b) 1 hour after the onset of subthreshold stimulation; c) After 1 hour 30 minutes of subthreshold stimulation; d) after 3 hours of subthershold stimulation; e) 40 minutes after the end of subthreshold stimulation; f) 4 hours 10 minutes after the end of subthreshold stimulation. Curves, from above downwards: contraction of ipsilateral semitendinosus, stimulation of the ipsilateral peroneal; stimulation of the contralateral peroneal; stimulation of the ipsilateral ulnar nerve ↑ , and contralateral ulnar nerve ↓, time marker (seconds). The figures show the strength of the stimulus current in terms of the centimeter scale of the induction coil.

caused an inhibition of the reflex contraction of the ipsilateral semitendinosus muscle (reciprocal inhibition), while stimulation of the ulnar nerve either had no effect, or else inhibited reflex contraction of the semitendinosi. After these test, nervous stimulation was applied to bring about the formation of a dominant. After avery 15-25 minutes, the stimuli were interrupted, and the test described above applied.

The criterion for the formation of a dominant in the flexor center of the spinal cord, was the appearance of an increase in the reflex contraction of the semitendinosi in response to stimulation of other afferent nerves (peroneal nerve of the contralateral side, and ulnar nerves of both sides).

RESULTS

In the first group of experiments, the dominant was developed in the flexor center of the hindlimbs by a long-maintained application of subthreshold stimulation (2-3 cm below threshold) of the peroneal nerve. The effect of these subthreshold stimuli, was to cause first an increase and then a decrease in the excitability and in the amplitude of contraction of the muscle.

As the dominant in the flexor center of the spinal cord developed, the inhibition of the flexor reflex by stimulation of the "test" nerves (peroneal nerve of the contralateral side and ulnar nerves of both sides) was first reduced, and then when the dominant had fully developed in the center of excitation of these nerves, instead of an inhibition, there was an increase in the amplitude of the flexor reflex.

As can be seen from the myograms (Fig. 1 a), recorded before the action of the subthreshold stimulation, the "supplementary stimulation of the afferent nerves, superimposed on the reflex contraction of the semitendinosus muscle, inhibited these contractions.

Stimulation of the same nerves separately, when the muscles were not otherwise stimulated to contract, produced no contraction of the ipsilateral semitendinosus. The myogram (Fig. 1,b), recorded 1 hour after the onset of subthreshold stimulation, shows that the "supplementary" stimulation of the ulnar nerves of both sides, separately, caused the ipsilateral semitendinosus to contract, while stimulation of the same nerves during reflex contraction of the semitendinosus caused an increase in the flexor reflex.

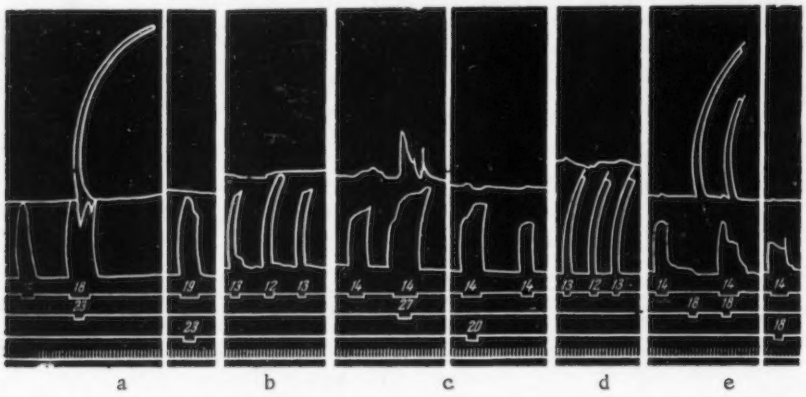

Fig. 2. Formation of a dominant in the flexor center of the spinal cord of the cat in response to prolonged threshold stimulation of the ipsilateral peroneal nerve. a,b) Initial condition; c) 20 minutes after onset of subthreshold stimulation; d) 1 hour after the onset of threshold stimulation; e) 3 hours after the onset of threshold stimulation. Traces as in Fig. 1.

During the time occupied by these changes, spontaneous rhythmic movements of the front and hindlimbs were observed quite often, in the form of automatic walking movements.

As can be seen from the next myogram (Fig. 1,c), the effect of a further 1 hour 30 minutes of subthreshold stimulation was to cause almost complete failure of inhibition of reflex contraction of the semitendinosus on stimulating the peroneal nerve of the contralateral side, while there was still, a more clearly shown increase in the reflex contraction of the muscle in response to the " supplementary" stimulation of the ulnar nerves of both sides.

When the dominant had become fully established in the flexor center, there was a complete elimination of the normal reciprocal relations between the centers of the antagonist muscles. Here, stimulation of the peroneal nerve of the contralateral side, applied during reflex contraction of the ipsilateral semitendinosus, caused an increase and not an inhibition of the flexor reflex.

The myogram (Fig. 1,d) recorded 3 hours after the onset of subthreshold stimulation, shows that stimulation of the peroneal nerve of the contralateral side during reflex contraction of the semitendinosus, causes no inhibition, but rather increases the strength of the reflex.

Stimulation of both ulnar nerves during reflex contraction increases the reflex considerably.

We were able, in these experiments, to observe the stability and inertia of the dominant focus, i.e., the

dominant was preserved for a considerable time, even after the cessation of the stimulus which originally caused it to be established.

The myogram (Fig. 1,e), recorded 40 minutes after the end of subthershold stimulation, shows a marked increased in the reflex contractions of the semitendinosus muscle, in response to the action of the "supplementary" stimulation of the contralateral peroneal and both ulnar nerves.

In Fig. 1,e , it can also be seen that there is an associated inhibition in the antagonist center during the formation of the dominant in the flexor center. Here , stimulation of the contralateral peroneal during contraction of the ipsilateral semitendinosus muscle, not only caused no contraction of the corresponding muscle, but on the contrary, caused it to be inhibited, while the reflex contraction of the ipsilateral semitendinosus was increased. "Supplementary"stimulation of the ulnar nerves caused an increase in the strength of the flexor reflex.

The next myogram (Fig.1,f) , was recorded 4 hours 10 minutes after the end of the subthreshold stimulation which led to the formation of the dominant. It can be seen that the "supplementary" stimulation of the contralateral peroneal again caused an inhibition of the reflex contractions of the semitendinosus, while stimulation of both ulnar nerves increased the reflex contraction of the muscle, thus indicating the great inertia of the dominant.

By applying "test" stimuli to various nerves during the formation of the dominant zone in the spinal cord, the power of the dominant zone to attract and to summate, at first, excitation arising from stimulation of afferent nerves associated with distant reflex arcs (ulnar nerves of both sides), and then, excitation arising in centers of afferent nerves associated with the reflex arc of the antagonist reflex (contralateral peroneal nerve) can be demonstrated.

More prolonged action of subthreshold test stimuli fails to cause an increase in reflex contraction. In this case, parabiotic changes may develop, and the dominant may pass into a state of inhibition . Stimulation of the contralateral peroneal during reflex contraction of the semitendinosus again induces reciprocal inhibition.

In the second experiment, a dominant was developed in response to the prolonged action of threshold and suprathreshold stimulation (2-3 cm of the induction coil scale above threshold).

During the development of the dominant by this means, phases were noted which resembled those found using subthershold stimulation.

With the threshold and suprathreshold stimuli, the dominant developed more rapidly, but was less stable. Parabiotic stages and the transition of the dominant to a state of inhibition occurred more rapidly in the latter case.

The myogram of Fig.2, a, recorded before the action of threshold stimulation, shows that stimulation of the contralateral peroneal during contraction of the ipsilateral semitendinosus inhibited reflex causes contraction of that muscle.

Stimulation of the ulnar nerve during reflex contraction, produced no effect. Increase in the strength of the stimulus (Fig. 2,b) caused an increase in the amplitude of reflex contraction, showing that there was no parabiotic stage. The drawing clearly shows, that with greater stimulation (12 cm on the induction coil) reflex contraction of the muscle was greater than with a weaker stimulation (13 cm on the coil).

The myogram of Fig. 2, c, recorded 20 minutes after the onset of the threshold stimulation, shows that stimuli applied to the contralateral peroneal nerve and ulnar nerves of both sides cause an increase in the contraction of the semitendinosus, thus indicating the formation of a dominant. But 1 hour after the onset of the action of the threshold stimulation, parabiotic stages occurred in this experiment.

Fig. 2, d shows the equalizing stage of parabiosis which then continues into the paradoxical phase.

When the threshold stimulation was continued, the dominant changed over into a condition of inhibition. The myogram of Fig. 2,e , recorded 3 hours after the onset of the threshold stimulation, shows a reduction in the reflex contraction of the muscle.

Stimulation of the contralateral peroneal and both ulnar nerves now causes an inhibition of the reflex contraction of the semitendinosus.

Consequently, during the development of a dominant focus in the flexor center of the spinal cord under

the influence of stimulation of an afferent nerve, many different phases occur, associated with change in the ability of the center to summate, and these phases are a) first an increase, and then a decrease in reflex excitability and in amplitude of contraction of the semitendinosus; b) reduction, and then complete disappearance of reciprocal inhibition; c) reflex contraction of the semitendinosus followed by an increased contraction in response to stimulating afferent nerves of remote reflex arcs (ulnar nerves of both limbs); d)spontaneous rhythmical movements of the front and hindlimb reproducing automatic walking movements; e) increase in the reflex contractions of the semitendinosus under the influence of stimulation of an afferent nerve reflex arc of an antagonist reflex (contralateral peroneal nerve), thus indicating distortion of the reciprocal relations between the centers of antagonists; f) development of parabiotic stages and the transition of the dominant into a condition of inhibition.

SUMMARY

Experiments were performed on decerebrate cats. The author studied the development of a dominant focus in the flexor center of the spinal cord in the action of subthreshold, threshold and suprathreshold excitations of the afferent nerve.

Experiments proved that in its development the dominant passes through a number of phases connected with the changes in the summation power of the center. After removal of the stimulations provoking the appearance of the dominant, the latter may continue for a long time (up to 4 hours), which demostrates its inertia. In response to threshold and suprathreshold stimulation of a definite intensity the dominant appears earlier, but is less stable. During futher stimulation it easily changes into inhibition, with development of parabiotic stages.

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** See English translation

GASEOUS EXCHANGE WHEN OXYGEN IS INHALED

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The problem as to how the gaseous exchange will alter when high concentrations of oxygen are inhaled originated immediately after oxygen had been discovered and the quantitative composition of the atmosphere determined.

The widespread use of oxygen in aviation and in medicine, both for treatment and prevention of anoxemia and as a general therapeutic measure, makes it important to study its effect on many physiological processes, and in particular on gaseous exchange.

However, there is considerable inconsistency in the results obtained by different workers. According to some [1,2,3], breathing almost pure oxygen does not affect the gaseous exchange, while others [4, 5, 6] claim that the gaseous exchange is reduced.

It must be noted that the great majority of workers consider that an increased barometric pressure depresses the oxidative processes only when the values are as high as 10-15 atmospheres.

The differences obtained when observing the effect of breathing oxygen on gaseous exchange and on the rate of oxidative processes are probably due to the fact that in different experiments the gas has been administered for different lengths of time, different amounts have been given, etc.

In the present work a study has been made of the gaseous exchange in animals breathing almost pure oxygen. It was measured in terms of the amount of carbon dioxide given out by the animal during a measured time.

METHOD

The experiments were carried out on 45 white rats. The method we used allowed us to study the gaseous exchange when breathing air with the normal oxygen content; the animal was then rapidly transferred into an atmosphere containing 93-95% oxygen, and, without interrupting the experiment, several determinations of the amounts of carbon dioxide given off at different times were made.

The animal was placed in small chamber through which air was sucked by a special pump; before entering the chamber, the air was made to pass through a washbottle containing alkali to remove the carbon dioxide.

The ventilation of the chamber was carried out so as to prevent any accumulation of carbon dioxide gas in it. The oxygen was supplied directly from an oxygen cylinder through a pressure-reducing valve, and the rate of flow was approximately the same as when normal air was used.

The percentage oxygen in the cylinder was determined by a gas analysis using Haldane's method. The carbon dioxide given out by the animal was absorbed in alkali placed in 2-3 washbottles through which the air or oxygen from the chamber was drawn, and it was measured by Winkler's method and referred to 100 g weight of the animal per hour.

Thus, our method enable variations in the rate of production of carbon dioxide to be studied, and made it possible to compare the amounts given out before, during, and after breathing pure oxygen, and to vary the duration of the experiment at will.

All the experiments were carried out after the animals had been fasting for some time; in between the experiments, they were given a normal complete food ration, consisting of oats, bread, meal, and milk.

More than 200 experiments were carried out in which the gaseous exchange was determined (in terms of carbon dioxide) under the following conditions: 1) when breathing normal air; 2) during the time they were breathing oxygen, 3) during the period of the so-called aftereffect, and 4) when breathing oxygen on two successive occasions.

RESULTS

When breathing air, there is a considerable variation in the gaseous exchange rate.

Thus, in some experiments, rat No. 1 gave off slighly over 120 cm^3 CO_2 per hour, while in other experiments, the same animal gave out more than 150 cm^3. Similar variations were found in other animals.

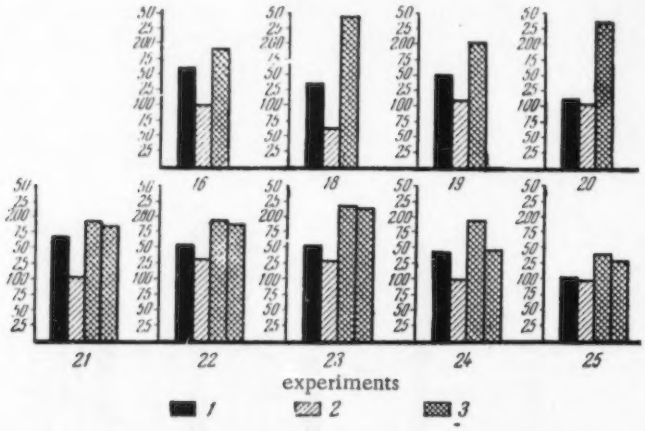

Fig. 1. Excretion of CO_2 (in cm^3) in rat No. 1 when breathing almost pure oxygen. 1) Initial condition; 2) during the breathing of almost pure oxygen; 3) when again breathing air.

On account of these very considerable variations in the gaseous exchange rate, which occurred under constant conditions, we did not take mean values even for separate animals, but found it more useful to make a comparison between the exchange rates in each case when oxygen was substituted for air.

The practical procedure was as follows. At first, the gaseous exchange rate under normal conditions, when air was passed through the chamber, was determined; then the measurement was repeated for oxygen, after which air was once more drawn through the chamber, and the respiratory exchange again determined.

Thus, in each separate experiment the gaseous exchange in a single animal was determined no less than 3 times.

Fig. 1 gives the results of experiment 1 for rats breathing almost pure oxygen, and it can be seen from the shaded column 2 that in most cases there was a relative reduction as compared with the initial value indicated by the black column 1.

There was a considerable reduction in the gaseous exchange rate in experiments No. 16,18, 19, 21, 24. In other experiments the reduction was less marked (experiments No. 22, 23); in experiments No.20 and 25 there was practically no alteration in the exchange rate.

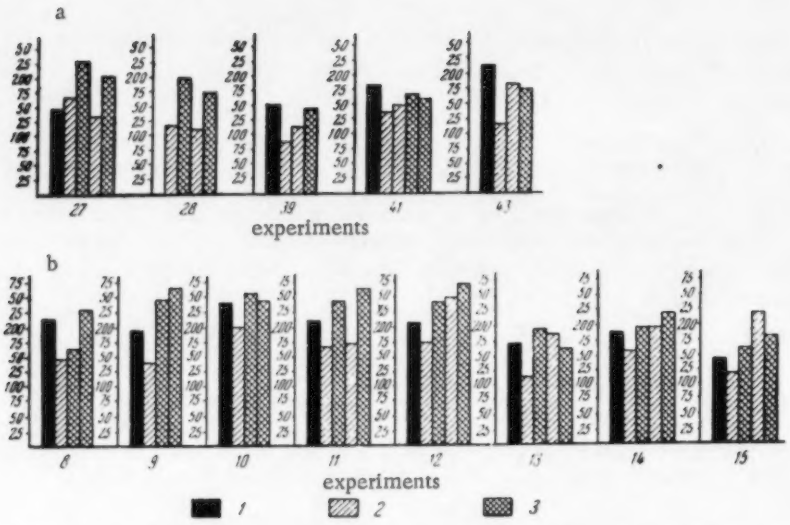

Fig. 2. Excretion of CO_2 (in cm^3) in rat No. 1 after breathing oxygen for two periods (a) of one hour (b) of two hours. 1) Initial rate; 2) when breathing almost pure oxygen; 3) after changing back to breathing air.

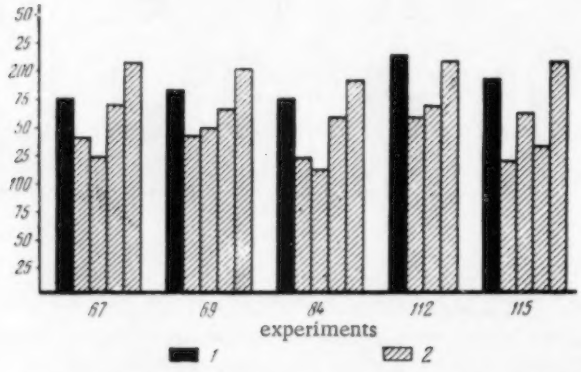

Fig. 3. Relationship between the excretion of CO_2 (in cm^3) and the length of time for which pure oxygen was breathed. 1) Initial condition; 2) during the breathing of almost pure oxygen.

It is important to note that not only rat No. 1, but also in the other 9 animals in whom the gaseous exchange rate was determined under these conditions, there was no case of the carbon dioxide production being raised, but on the contrary reductions were observed from 20 to 47 %.

From Fig. 1 it can be seen that during the aftereffects of breathing oxygen (i.e., after the animal has changed over from breathing oxygen to breathing air containing the normal amount of oxygen) there was usually an increase in the amount of carbon dioxide produced. This can be seen from the results of experiments No. 16, 19, 20, 23, and 24, as shown by shaded columns 3.

From these results it may be concluded that when the animals are transferred after breathing oxygen for a 30-minute or for a 1-hour period to breathing air, there is an increase in the amount of CO_2 given off.

Increase in the production of carbon dioxide during the period of afteraction, continued for not more than 1 hour 30 minutes, after which the rate returned to normal.

If the amount of carbon dioxide given off by the animal is interpreted as indicating the oxygenation of all organic substances and taken as an index of the rate of the catabolic processes in the organim, then it can also be said that the rate of the oxidation processes is loweres during the breathing of pure oxygen, and rises once more when it ceases.

In the next experiments, the gaseous exchange was determined in rats which spent two one-hour or half-hour periods breathing oxygen. For this purpose, first the amount of carbon dioxide given off during the breathing of normal air was determined, the same quantity was then measured when breathing oxygen, then again when breathing air, and once more when breathing oxygen, and so on. The animals were left for the whole time in the chamber, and the gaseous exchange rate determined every 30 minutes, or every hour.

The results obtained are given in Fig. 2, from which it can be seen (experiments No. 11, 27, 28 and 39) that during the period of breathing oxygen (shaded column 2) over a period of 30 minutes or 1 hour, the production of carbon dioxide was markedly reduced, as compared with the normal value obtained when breathing normal air (black column), and that it was increased when the oxygen was withdrawn, both after the first and after the second application.

No appreciable difference was found between the cases when the oxygen was given once or twice. Both in the first and in the second oxygen breathing sessions, there was a reduction of from 20% upwards in the amount of carbon dioxide given off.

The next experiments concerned the gaseous exchange in rats as affected by the duration of the period for which they breathed almost pure oxygen.

From the published results, and from our observations [7, 8, 9] it is known that when oxygen is given in high concentrations, it becomes toxic and causes not only inflammation of the upper respiratory tract, but also of the lungs.

We have shown [8,9] that if frogs, mice, or rats are maintained for long periods in almost pure oxygen, even at normal atmospheric pressure, they die after quite a short time. In these cases, death occurs on the 5th or 6th day, as a result of pulmonary inflammation and edema.

From our observations, we can see that as far as white mice and rats are concerned, a daily sojourn of 4 hours per day for 3-4 months in oxygen causes no organic change in the lungs, and is almost harmless, causing only transitory functional changes, including alteration of the heart rate, respiration, blood gases, etc.

It seemed important to us to find how the gaseous exchange changes in response to prolonged periods in pure oxygen.

Our method made it possible to keep the animals in almost pure oxygen for a long period, and to determine the carbon dioxide, as an index of the gaseous exchange, at any time intervals. Usually, in this set experiments, measurements of the carbon dioxide were made hourly and were referred to 100 g weight of the animal.

For the experiment, 14 healthy rats were used. Over 120 determinations of carbon dioxide were made.

The dependence of the gaseous exchange when breathing pure oxygen on the duration of its action are shown in Fig. 3, where it can be seen that in the groups of 5 or 4 experiments shown, Nos. 67, 69, 84, 112, and 115 indicate that the amount of carbon dioxide excreted changed as described below; results for the other experiments are not given, because their results were the same.

During the first and second hour of breathing oxygen, the amount of carbon dioxide gas given off was reduced (compare the shaded with the black columns), while in subsequent one-hour periods, usually in the third hour, the amount attained the initial value. After 3 hours of breathing oxygen, the amount of carbon dioxide gas given off, as compared with the initial (control) value when breathing air, was increased by 15-20 %.

In control experiments, when the animals breathed air for a corresponding period, no such regular changes in the gaseous exchange rate ensued.

The increased production of carbon dioxide after a 3-4 hour period of breathing oxygen may be due to the greater motor activity of the animals, and to the increase in respiration which occurred frequently.

It is not impossible that the increase in the oxidative processes occurring during prolonged breathing of oxygen is due to its stimulating action on the respiratory tracts. Evidence for this is afforded by the indisputable fact which has often been reported that when breathing high concentrations of oxygen, there is a hyperemia of the upper respiratory passages.

From a large number of experiments and by using a method which allowed a comparison to be made in a single experiment of the amount of carbon dioxide given off when breathing air and oxygen respectively, we may state that when breathing 93-95 % oxygen, there is a reduction in the gaseous exchange rate, which then increases somewhat 1-2 hours after a return to normal air breathing has been made.

SUMMARY

Gaseous exchange was studied in white rats breathing almost pure oxygen. The carbon dioxide output during known time intervals was taken as a measure of the gaseous exchange rate.

This method was used to study changes in the carbon dioxide output, and to compare the values before and after breathing oxygen, and to determine the aftereffect. More than 20 experiments were performed.

In some experiments, almost pure oxygen was breathed, and the gaseous exchange, as measured by the carbon dioxide output, decreased by more than 20%. However it again rose during the period of the aftereffect, which lasted not more than 1½ hours.

Measurements on gaseous exchange were made in healthy rats breathing almost pure oxygen for 3-4 hours. In all, 120 experiments were performed. It was shown that when oxygen was breathed for more than 3 hours, there was some increase in the gaseous exchange rate. This was associated with the stimulating effect of oxygen in the respiratory passages.

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THE PART PLAYED BY THE MUCOUS MEMBRANE OF THE ACCESSORY NASAL SINUSES IN REGULATING BREATHING

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Respiration rate is to some extent controlled by reflexes originating from the mucous membrane of the upper part of the respiratory tract [2, 3, 4, 7, 8, 15 and others]. It may be taken as established that stimulation of the trachea, larynx, and nose by various stimuli including a stream of air is a factor which has an important effect on respiratory movements. In the works of the Pathophysiological Laboratory of the Scientific Research Institute of the Ear, Nose, and Throat, it has been shown that reflex influences from the upper respiratory tract are of particular importance, especially in pathological conditions, in regulating the activity of various organs and systems, in particular the heart, stomach, kidneys, etc. [5, 6, 9, 11].

Less attention has been paid to the part played by the mucous membrane of the accessory nasal sinuses in regulating respiration.

Some workers [1, 10, 12] have indicated the possibility of reflex control of the respiration from the mucous membrane of the accessory nasal sinuses. Others deny this [13 and others]. It may be that these differences have developed through work having been done on animals in acute experiments using different kinds of anesthetics and different depths of anesthesia.

Our present task is to determine the extent to which the mucous membrane of the frontal sinus takes part in reflex regulation of the respiration.

METHOD

Chronic experiments were carried out on dogs, no anesthetic was used, and there was no painful stimulation. The frontal bone was trepined over the frontal sinus, on the left and right sides, under ether anesthesia. A fistula was established by introducing a duralumin tube and sealing it hermetically into the opening in the bone. The tube was fixed into the bone by means of threaded sleeve over which the skin was sewn. After 6-7 days from the time of the operation, when all inflammatory changes had subsided, the animal was ready for the experiment. A current of air, and mechanical, chemical and thermal stimuli were applied to the mucous membrane.

The animal was placed on a stand having a special adaptation for holding the trunk and head still. A pneumograph was used to record the respiration on a kymograph. The cover was then unscrewed from the fistula, and the mucous membrane of the frontal sinus stimulated. Altogether 220 experiments on 7 dogs were carried out.

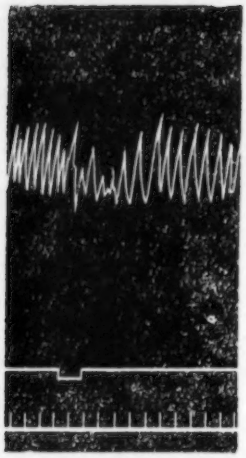

Fig. 1. Changes in the respiration in the dog Soroka in response to mechanicall stimulation of the mucous membrane of the frontal sinus. Curves, from above downwards: respiration; stimulus marker; time marker (1 second).

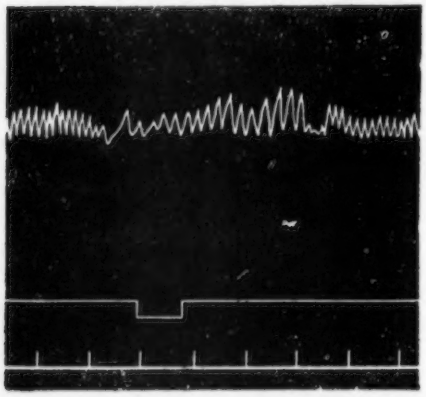

Fig. 2. Changes in respiration in the dog Sharik in response to cold stimulation of the mucous membrane of the frontal sinus. Curves as in Fig. 1.

RESULTS

In 29 experiments the effect of mechanical stimulation of the mucous membrane of the frontal sinus on the respiration was determined. Various parts of the mucous membrane of the sinus were touched by introducing a fine metal wire through the fistula. In order not to cause damage to the membrane, the end of the wire was covered with a fine layer of cottonwool.

In 19 experiments, mechanical stimulation of the mucous membrane of the frontal sinuses caused a slowing of the respiration rate and an increase in the amplitude of the respiratory movements, while in 5 experiments there was no reaction, and in 5 others the respiration rate was raised and the amplitude reduced. It must be noted that stimuli which were above the level of the tactile sensibility, caused a motor response to the pain caused, and this led to errors in recording respiratory movements. This probably accounts for failure to obtain results in many experiments. Thus, it must be supposed that, as a rule, mechanical stimulation causes a change in the rate and amplitude of respiratory movements (Fig. 1).

To study the action of thermal stimuli on the frontal sinus, we introduced 0.2-1 ml of water at temperatures from 5 to 50°. From the results of 48 experiments, it was found that the effect of both cold and hot water was the same as that of mechanical stimulation (Fig. 2).

As chemical stimuli, in 28 experiments we used a solution of penicillin (150,000 units per ml), and in eighteen, a 0.1 N solution of hydrochloric acid, and in a further eighteen, a 0.1 N solution of caustic soda. In each case, 0.2-1 ml of the substance was introduced into the sinus. The temperature of the solutions was between 16 and 18°. In all the experiments, there was an increase in the amplitude and a decrease in the rate of respiration (Fig. 3, a).

The most revealing experiments were those in which the mucous membrane of the frontal sinus was stimulated by air, i.e., by the stimulus which is effective under normal conditions. The air was introduced into the sinus through the fistula tube, under control of a manometer. The pressure varied from 20 to 140 mm of mercury. In spite of the different stimulus strengths, in 38 out of the 54 experiments there was an increase in amplitude and decrease in rate of respiration. Only in 9 experiments did the reverse change occur, while in 7 there was no reaction.

When using oxygen as a stimulus, controlled by the manometer, in 16 out of the 25 experiments there was some change in the amplitude of respiration (either an increase or a decrease, while in 2 experiments there was no change; reduction in the respiration rate was found only in 10 experiments, there being no change in the remaining 15. Changes in the amplitude were less marked than in the case of the other stimuli.

Thus oxygen is a considerably less potent stimulus for the receptors of the mucous membrane of the frontal sinus than is air, and the effect is chiefly on the amplitude rather than on the rate of respiration (Fig. 3, b).

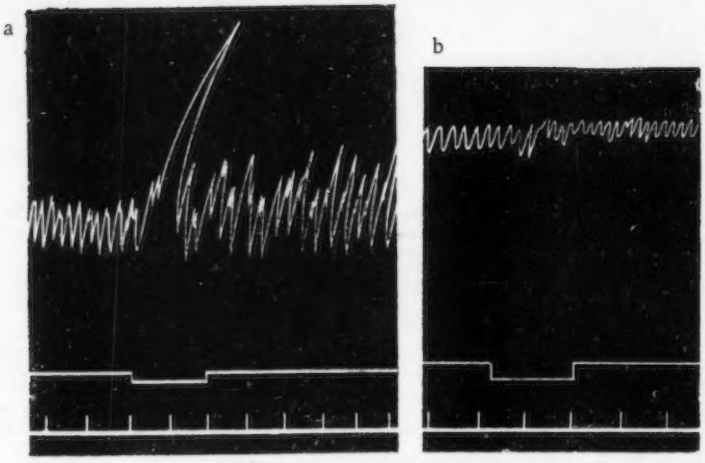

Fig. 3. Changes in respiration (a) in the dog Umka, on introducing penicillin solution into the frontal sinus, (b) in the dog Soroka in response to oxygen on the same mucous membrane. Traces as in Fig. 1.

From these results it may be concluded that in the mucous membrane studied there are chemo-, baro- and thermoreceptors. All the types of sitimuli applied to the mucous membrane of the accessory sinuses have some effect on the respiration.

The results we have obtained do not agree entirely with other published accounts [1, 4]. Thus, A. A. Atkarskaya [1] reports that with mechanical stimulation of the mucous membrane of the frontal sinus, the respiration rate is raised, and stimulation with water at a temperature of 3 or 4° has no effect. Chemical stimuli such as ether and ammonia solution have no appreciable effect on respiration, and cause only a reaction of the animal in response to the pain induced.

As far as stimulation of the mucous membrane of the sinuses with air is concerned, our results agree with those of A. A. Atkarskaya with respect to the slowing of respiration rate, but do not agree with her findings on the amplitude of the respiratory movements. She reports a reduction, whereas, in most cases, we observed an increase in amplitude. Reduction in the amplitude and rate of respiration in response to an air stimulus should, according to A. A. Atkarskaya, be interpreted as the result of an inhibition, which is usually a characteristic of the trigeminal nerve.

However, E. N. Pavlovskii and Shpiss have shown that stimulation of the trige minal nerve causes an increase in the depth of respiration, a result which agrees with our findings. Kerekes[12] has found that weak stimuli cause an increased and strong stimuli a decreased ventilation; the effect depends upon the strength of the stimulus.

It is not easy to compare our results with the others reported above, because the experiments were carried out by different methods.

From the results obtained we may conclude that the accessory nasal sinuses take part in the regulation of respiration.

SUMMARY

Chronic experiments were performed on dogs with a fistula established in the frontal sinus. The effects on respiration of mechanical, chemical, and thermal stimuli, as well as of air and oxygen currents were investigated.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PHYSIOLOGY

THE EXPERIMENTAL PRODUCTION OF A NEUROSIS IN MONKEYS BY CHANGING THE DIURNAL RHYTHM

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The higher nervous activity of monkeys is distinguished by its great complexity and it has many special features which enable it to withstand traumatic influences. For this reason, attempts to induce neurosis in monkeys by means of the ordinary methods used with success in dogs have been inconclusive. A neurosis was developed only in monkeys with a weak type of nervous system and with inert nervous processes, in response to very complicated and difficult tasks [1, 3, 6, 8].

Greater success attended D. I. Miminoshvili's [7] attempt to induce a neurosis in monkeys by the use of conflict, on the basis of natural stimuli addressed to different cortical and subcortical centers mutually inducing each other.

In the present research an attempt was made to produce a neurotic state by changes in the diurnal rhythm, or stereotype.

The diurnal rhythm, which has been developed on the basis of a whole series of daily repeated stimuli, is a dynamic stereotype in which the leading role is played by conditioned reflexes to the time of action of these stimuli. By changing the place and time of action of the components of the diurnal stereotype, i.e., by transferring the lighting up and feeding to the hours of night or by repeating them twice in the 24 hours, it is possible to obtain a new reflex to time, in the form of a distorted or biphasic diurnal rhythm [1, 9, 11, 12]. If the diurnal stereotype is changed to one directly opposite without waiting for the reorganization of the physiological functions in accordance with the new regime to be completed, then this break may cause disturbances in the working of the cerebral cortex.

EXPERIMENTAL METHOD AND RESULTS

The experiment was originally carried out on 3 monkeys of the species Papio hamadryas: Bronenosets, Nosorog and Dog. In all three monkeys, motor alimentary conditioned reflexes were induced to a simple stereotype, a light and a bell as positive conditioned signals, and a tone as a differential signal.

By the use of special tests it was ascertained that all 3 monkeys were of the strong type, but that Bronenosets was weaker than the others. In Nosorog the excitation process was predominant. As regards the mobility of their nervous processes Dog and Bronenosets were inert and Nosorog mobile.

After this had been done, the monkeys were transferred to a special artificial diurnal regime in which the stimuli were arranged in the opposite order before the monkeys were able to reorganize themselves to the new regime assigned to them. Alternation of the artificial diurnal regimes took place in the following order: a natural diurnal regime was replaced by a distorted regime (rest and darkness by day and illumination and

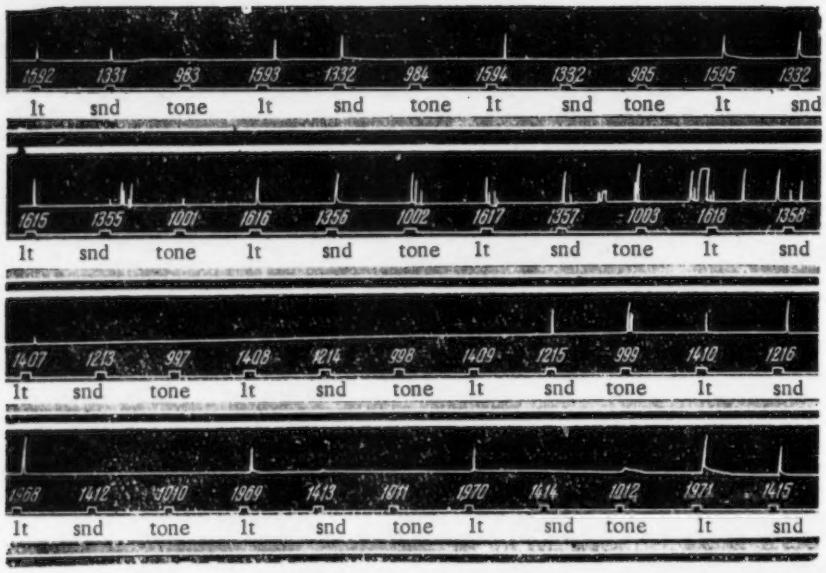

Fig. 1. Changes in the conditioned reflex activity of monkeys under normal conditions and during the development of a neurosis. a) Under normal conditions; b) derangement of the nervous activity leading to excitation; c) derangement of nervous activity leading to inhibition; d) pardoxical phase. Legend from above down: pressure on the lever; stimulus marker; time marker in seconds.

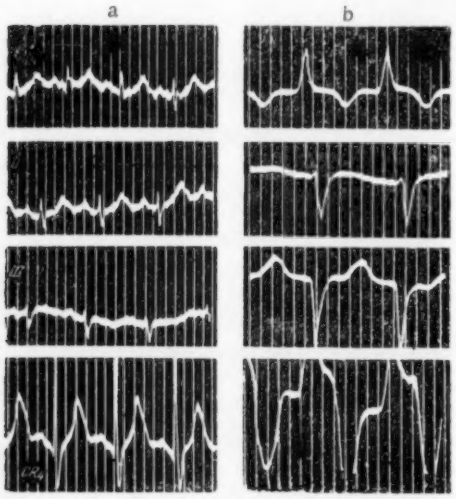

Fig. 2. Changes in the electrocardiogram in Nosorog. a) Before the illness; b) during illness; I, II, III) standard leads.

feeding by night). As soon as the monkeys had begun to adapt themselves to the new conditions, the regime was changed to one of biphasic days (2 "days" and 2 "nights", each of 6 hours' duration, in the course of 24 hours), followed by biphasic days again, but with the opposite sign, i.e., where there had been a "night", a "day" was substituted, and vice versa. Illumination for 24 hours continuosly was then replaced by darkness for 24 hours continuosly, and so on.

As a result of this traumatization, all three monkeys developed a neurosis after 2 months of the experiment. Their conditioned reflex activity was severely deranged. The monkey Nosorog was very excited, and pressed several times on both the positive and the differential signal lever (Fig. 1,b). Dog sat in a corner all the time, not reacting to signals, and only at the end of the experiment did he press on the lever from time to time, although he did not come near the bait (Fig. 1,c). Bronenosets showed a well marked paradoxical phase, for the light was followed by a stronger pressure on the lever than the bell (Fig. 1, d). This neurosis continued for a few months after the monkeys had been transferred back to their normal regime.

The initial kymograms of conditioned reflex activity were identical in all the monkeys (Fig. 1,a).

Nosorog took part in the experiment without showing any signs of derangement of cardiac activity (Fig. 2,a). Two months after the end of the experiment the monkey became relatively immobile and sluggish and had frequent bouts of trembling. A few days later she began to eat, and lay motionless on the floor of the cage all the time.

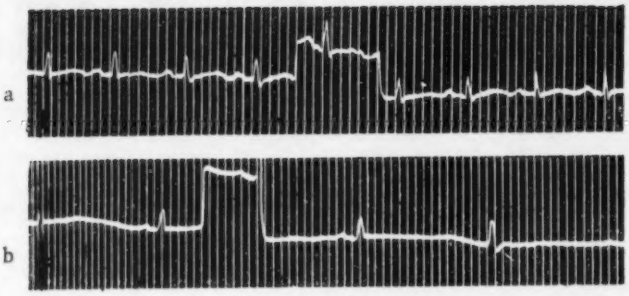

Fig. 3. Changes in the electrocardiogram in Khisar (lead II). a) Initial heart rate 171 per minute; b) bradycardia as a result of neurosis; heart rate 70-77 per minute.

The electrocardiogram at this time showed a negative T-wave and the presence of a QS peak; the QRS complex was drawn out, in the CR_4 chest lead a deep negative T-wave was observed, and the S—T interval was elevated 6 mm above the isoelectric line (Fig. 2,b). Four days later the monkey died with signs of acute coronary insufficiency. At autopsy circulatory disturbances were found and micronecroses were present in the heart. No visible abnormalities were found in the other organs.

Subsequently, when a neurosis was induced in another group of monkeys by disturbance of the diurnal stereotype, in addition to coronary insufficiency other forms of disturbance of cardiac activity were observed —the so-called "vagus neurosis". Under these circumstances the bradycardia reached 75-80 beats per minute, with an initial normal heart rate of 170-180 per minute (Fig. 3).

Such a strong influence of changes in the diurnal rhythm on the cardiovascular system of the monkeys is probably accounted for by the fact that these changes constituted the elements of a conflict. When the nerve cells, by virtue of the conditions created, had fallen into a state of inhibition, stimuli were put into action leading them into a state of excitation, and vice versa.

These changes in the diurnal rhythm were continued for long periods of time, each day, and not briefly during the experiment in the chamber.

Any interference with the stereotype and the intrinsic rhythm of work is tolerated badly, both by animals and by man. Experiments have shown [4, 5] that heterorhythmic stimuli are tolerated badly by the nervous system, and lead to neurosis in dogs and rabbits. According to the findings of M. B. Tsukker and L. M. Khvan [10] and of Pierach [13], work on different shifts often leads, in man, to neurosis and to consequent somatic disease.

It may be postulated that the neuroses and attendant diseases which we induced by interference with the diurnal stereotype are close in their nature to certain clinical disturbances encountered in man.

SUMMARY

Neurosis was experimentally induced in Papio hamadryas through systematic interference with their diurnal routine (for the period of 2 months) by varying the periods of ilumination and feeding.

This neurosis took the from of disturbances of the conditioned reflex activity and of the appearance of somatic pathology: coronary insufficiency, micronecroses in the heart (in one monkey) and cardiac "vagus neurosis" with pronounced bradycardia.

Neurosis thus produced is of special interest, since by its etiology and complications, it is very similar to disorders occurring in man.

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THE ROLE OF THE BLOOD ADRENALIN AND OF CARDIOSCLEROSIS IN THE MECHANISM OF PAROXISMAL TACHYCARDIA

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Attacks of paroxysmal tachycardia are often observed in persons with heart disease, cardiosclerosis and other diseases of the cardiovascular system. This type of attack is known to be based on the appearance of a hypertonic source of excitation in the myocardium, which temporarily becomes the pacemaker of the cardiac rhythm. However, the factors which lead to the formation of the focus of heterotopic excitation, convert it into the pacemaker of the cardiac rhythm, and thereby provoke attacks of paroxysmal tachycardia, are in many respects unexplained.

In order to study this problem further, in long term experiments we produced attacks of paroxysmal tachycardia in dogs with experimental heart lesions.

EXPERIMENTAL METHOD

A graded and permanent experimental lesion was created by constriction of the aorta immediately above the valve. Under these circumstances the cross section of the aortic orifice was reduced to one quarter its original size. The ligature producing stenosis of the aorta was passed through a polyvinyl chloride tube and did not damage the wall of the aorta. Four dogs with compensated aortic stenosis were kept under observation for 12-18 months after operation.

Before and after the creation of the heart lesion in these animals, the changes in the cardiac activity arising after the intravenous injection of adrenalin (0.5 ml of a 1: 1000 solution) were recorded electrocardiographically. A rise in the arterial pressure is known to develop as the result of the direct vasoconstrictor effect of adrenalin. The action of the increased pressure on the pressure receptors in the aortic and carotid sinus zones leads to intensification of the signals from these receptors, to excitation of the vagus nerve center and to reflex bradycardia. After division of the vagus nerves, the intravenous injection of adrenalin in our experiments, as in those by other research workers [1], did not cause bradycardia.

The reflex bradycardia caused by adrenalin was expressed as a fall in the heart rate from 100-72 to 60-42 beats per minute; it lasted 2-3 minutes and was replaced by tachycardia. On this background of bradycardia, attacks of paroxysmal tachycardia regularly developed in the dogs with the heart lesion, and these attacks formed the object of study in the present research.

EXPERIMENTAL RESULTS

Against the background of adrenalin-induced bradycardia in the dogs, extrasystoles and attacks of paroxysmal tachycardia were observed. In evaluating these phenomena, it must first of all be emphasized that extrasystoles were present during bradycardia not only in the dogs with the heart lesion, but also in the same

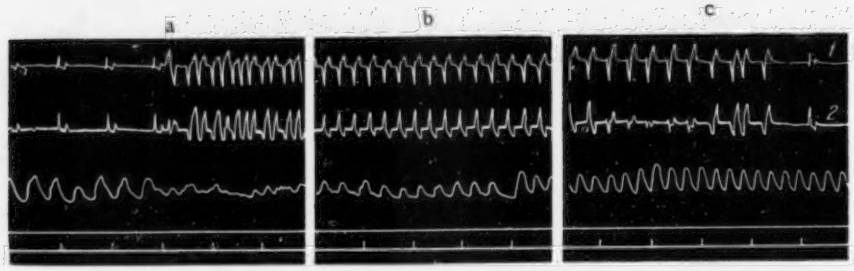

Fig. 1. Electrocardiogram and pneumogram at the beginning (a), in the middle (b) and at the end (c) of an attack of paroxysmal tachycardia in a dog. 1) ECG, lead I; 2) ECG, lead III.

animals before operation, but attacks of paroxysmal tachycardia were observed only in animals with a heart lesion and were never found in normal animals.

Extrasystoles developed in normal animals in roughly half the experiments, and was expressed as the appearance of single and sometimes of groups of left ventricular or right ventricular extrasystoles. After the creation of a heart lesion, the extrasystoles on the background of the adrenalin bradycardia were sharply intensified; they were observed in all the experiments, occurring in the 1-4 months immediately after production of the lesion, and were expressed as groups of right ventricular and left ventricular extrasystoles, alternating with each other. At times the extrasystolic contractions followed one after the other, and then it was possible to speak of a ventricular rhythm. The electrocardiogram has a mixed appearance, for on it right and left ventricular extrasystoles alternated with normal cardiac cycles.

The appearance of heterotopic sources of excitation in the myocardium and the development of extrasystoles are evidently due to the fact that in the first phase of its action adrenalin had a dual effect on the heart.

Reflexly, and by its action on the vagus nerve center, adrenalin causes vagal inhibition of the automatism of the sinus node, and thus makes possible the emergence of potential sources of heterotopic excitation. On the other hand, the direct action of adrenalin on the heart is not only positively chronotropic, but also consists of increasing the excitation of the myocardium. Under these circumstances the influence of the vagus nerve extends to a far greater degree to the sinus node than to the myocardial cells of the ventricles, whereas the direct action of adrenalin is addressed equally to both components of the system. As a result, when adrenalin is present in the blood, a situation is unfolded in which the normal pacemaker of the cardiac rhythm is inhibited and the potential sources of cardiac automatism pass into a state of excitation — extrasystoles are produced. After the reflex inhibition of the sinus node disappears and the adrenalin bradycardia is replaced by tachycardia (second phase of the action of adrenalin), the extrasystoles quickly disappear.

Attacks of paroxysmal tachycardia of left ventricular origin were observed in all four dogs under investigation for a long time. At first the attacks arose $3\frac{1}{2}$-4 months after the creation of the heart lesion, they appeared suddenly 50-70 seconds after the beginning of the adrenalin bradycardia and lasted from 35 to 55 seconds. The left ventricular rhythm during the attacks was over 200 beats per minute (204, 210, 214). The attacks ceased just as suddenly as they began. In the few seconds immediately before the attack began or after it ended, isolated left ventricular extrasystoles were observed (Fig. 1, a-c).

An essential feature was that in our experiments the attacks of paroxysmal tachycardia arose, not episodically as in the majority of experimental investigations previously published [2, 3 and others], but in a form regularly reproducible from one experiment to the next.

According to the most reliable view of the common origin of extrasystoles and paroxysmal tachycardia, the main rola in the mechanism of these phenomena is played by the appearance in the myocardium of a focus of heterotopic excitation, which, under suitable conditions (a lowering of the automatism of the sinus node, an increase in the excitation of the myocardium and so on), temporarily becomes the pacemaker of the cardiac rhythm. In this connection it was important to determine whether or not signs of cardiosclerosis developed in the muscle of the hypertrophied left ventricle of the dogs with experimental aortic stenosis, since areas of myocardium situated in the immediate proximity of cardiosclerotic foci may act as sources of heteropic excitation in paroxysmal tachycardia.

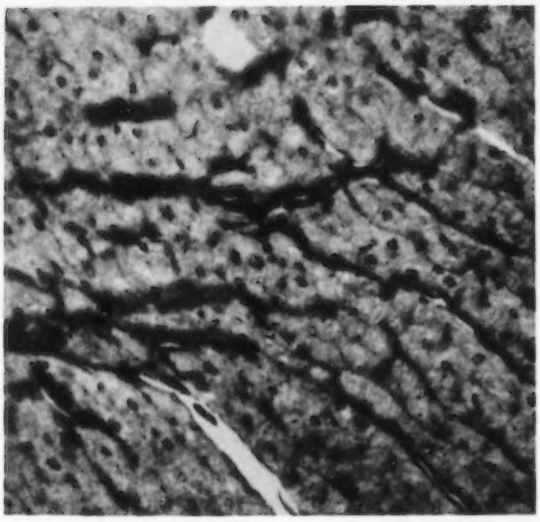

Fig. 2. Marked cardiosclerosis in the myocardium of the left ventricle of a dog with an experimental heart lesion. Thick bands of collagen fibers are seen, situated between the muscle bundles.

The subsequent morphological examination of the hearts of the dogs with aortic stenosis, in which attacks of paroxysmal tachycardia were observed, showed that hypertrophy of the left ventricle in these animals was combined with the development of a well-marked cardiosclerosis. On investigation of the heart of the dog Pushok, for example, 8 months after the creation of the lesion, the findings were as follows: absolute weight of the heart—201 g, relative weight of the heart— 0.0011, i.e., 55-60 % greater than normally.

On histological examination of the heart, hypertrophy of the muscle fibers was found, mainly in the wall of the left ventricle. There was predominance of thickened muscle fibers with large, light, elongated nuclei. The borders between the muscle fibers were ill-defined. Alongside the hypertrophied fibers, thinned, atrophic muscle fibers with small hyperchromic nuclei were seen. On staining with picrofuchsin, well-marked cardiosclerosis was found, in the form of proliferation of thick collagen fibers between the muscle bundles (Fig. 2). Perivascular sclerosis with thickening of the tunica media of the arteries and the development in it of collagen fibers were found. Basically similar results were obtained on examination of the hearts of the other animals.

Thus in the dogs with experimental aortic stenosis, side by side with hypertrophy of the myocardium was observed the development of cardiosclerosis, and against a background of adrenalin-induced bradycardia arose attacks of paroxysmal tachycardia.

When regarding the areas of myocardium stimulated by the cardiosclerotic process as potential sources of heterotopic excitation, it must be supposed that during adrenalin-induced bradycardia, on account of reflex inhibition of the automatism of the sinus node, and also as the result of the direct excitatory action of adrenalin on cardiac muscle, these potential sources of heterotopic rhythm in the myocardium of the left ventricle become its real sources — an attack of paroxysmal tachycardia then supervenes.

Situations in which, against a background of some degree of cardiosclerosis, there is a simultaneous lowering of the automatism of the sinus node and an increase in the excitation of the myocardium are evidently also possible in clinical practice, for a rise in the blood adrenalin is frequently observed in man and is an indispensible component of states such as anger, fear, pain and physicial strain. It is very probable that these cortically induced states, arising against a background of cardiosclerosis, from time to time provoke the onset of attacks of paroxysmal tachycardia or extrasystoles in man.

SUMMARY

Extrasystoles were observed in normal dogs against the background of reflex bradycardia caused by the intravenous administration of adrenalin. Three - four months after the creation of experimental aortic stenosis attacks of left ventricular paroxysmal tachycardia appeared in the same animals after the administration of the same dose of adrenalin. Three factors play an important role in the appearance of these attacks: 1) vagal inhibition of the automatism of the sinoatrial node; 2) direct action of adrenalin, increasing the myocardial excitability; 3) stimulating effects of cardiosclerotic foci on definite areas of the left ventricular myocardium.

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THE INFLUENCE OF THE EXTRACARDIAC NERVOUS SYSTEM ON THE FUNCTIONAL STATE OF THE HEART IN EXPERIMENTAL MYOCARDIAL INFARCTION

COMMUNICATION II. THE EFFECT OF REPEATED ELEVATION OF THE TONUS OF THE VAGUS NERVE CENTER ON THE COURSE OF EXPERIMENTAL MYOCARDIAL INFARCTION

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Laboratory investigations on dogs with experimental myocardial infarction have shown that an increase in the tonus of the vagus nerve center by the injection of adrenalin against a background of the action of morphine, or stimulation of the peripheral cut end of the vagus nerve by means of an induction coil, strengthening the contractions of the ventricles of the heart, leads to a lack of correlation between the strength of the contractions and the blood supply of the myocardium. The ensuing functional overload brings about the more rapid and extensive development of myocardial infarction of the ventricles of the heart [8, 9]. The importance of the factor of functional overload is confirmed by the work of several authors [1, 2, 11, 12].

Studies of the pathogenesis of myocardial infarction have shown that in the first days after ligation of the descending branch of the left coronary artery, polytopic ventricular extrasystoles develop, to a large extend associated with the influence of the vagus nerve on the heart [8]. The role of the vagus nerve in the production of extrasystoles has been pointed out by other workers [3, 6, 13, 15, 16].

In the most recent investigations in the laboratory it has been found that, during the first 5-6 days after the onset of experimental myocardial infarction, an increase in the tonus of the vagus center does not cause a slowing of the rhythm, but increases the extrasystoles of the ventricles of the heart. On the 5th-6th day after operation, the inhibitory action of the vagus nerve on the heart is restored, and this coincides with disappearance of the extrasystoles [10]. A change in the reactions of the heart in response to stimulation of the vagus nerve, in association with myocardial damage, has also been observed by other workers [5, 6, 14].

These findings enabled an approach to be made to the study of the action of the heart and of the effect of an increase in the tonus of the vagus center on the heart in the later stages of development of experimental myocardial infarction in dogs of different ages. This question is dealt with in the present paper.

EXPERIMENTAL METHOD

Chronic experiments were conducted on 5 healthy dogs of different ages. In order to produce an experimental myocardial infarct in these animals, the descending branch of the left coronary artery was ligated. The operations were performed in sterile conditions under morphine-urethane or morphine-kemithal anesthesia. The development of an infarct was confirmed electrocardiographically and by subsequent morphological examinations. The tone of the vagus center was raised in the chronic experiments by injection of morphine and calcium chloride.

Morphine was injected subcutaneously (1-2 ml of a 1% solution), and the calcium chloride intravenously in the form of a 10% solution, the dose being 0.05 g/ kg body weight. The chronic experiments on the dogs Donor and Lysyi concluded with acute experiments under morphine-kemithal anesthesia, in which both vagus nerves were divided and their peripheral cut ends were stimulated by an induction current.

Recordings were made of the electrocardiograph of all the experimental animals using the 3 standard leads, and using lead II for prolonged observations on the action of the heart. Needle electrodes were introduced subcutaneously. The heart rate was counted and the heart sounds and murmurs auscultated during the experiments. In the chronic experiments the dogs were made to stand on the apparatus, and in the acute experiments the arterial pressure was recorded with a mercury manometer. The peripheral cut end of the vagus stimulated by means of a DuBois-Reymond induction apparatus from a 5v accumulator.

EXPERIMENTAL RESULTS

We give a brief description of the results of the experiments on the dogs Donor, Volchok, Starik, Lysyi and Rek.

The dog Donor. Male, weight 28 kg, age 4 years. Mobile, balanced. on January 20, 1958 the descending branch of the left coronary artery was ligated in its middle third. The dog was kept under observation for 114 days. After operation, polytopic ventricular extrasystoles appeared, lasting 8 days. Raising the tonus of the vagus center on the 9th day caused considerable left ventricular extrasystoles. On the 11th day an attack of paroxysmal ventricular tachycardia was observed, which lasted about 24 hours. On subsequent days an increase in the tonus of the vagus nerve center led to slowing of the cardiac contractions; there were no extrasystoles. Throught the whole period of observation on the dog there was no change in its behavior. The heart rate was between 100 and 120 beats per minute. In the acute experiment, in response to stimulation of the peripheral cut end of the vagus nerve on the 114th day a slowing of the rate was observed, with isolated extrasystoles, which continued after cessation of the stimulation.

The heart at autopsy: in the anterior wall of the left ventricle there is a whitish scar, measuring 5 × 5 cm; thickness of the left ventricle 1.5 cm, of the right ventricle 0.5 cm.

The dog Starik. Male, weight 16 kg, age about 12 years. Tranquil, inhibited, sluggish. The dog remained under observation for 42 days. On March 26, 1958 the descending branch of the left coronary artery was ligated in its upper third. Only 40 minutes after operation low-voltage extrasystoles appeared; the heart rate quickened. On the 7th day , intravenous injection of calcium chloride against a background of the action of morphine led to continuous extrasystoles which lasted one hour. Electrocardiogram on the 9th day: T_1—negative, $T_{2,3}$—above the isoelectric level, T_3—high, coronary , monophasic curve. Elevation of the tonus of the vagus center on the 10th day was accompanied by extrasystoles and by atrioventricular block. The monophasic curve persisted in this experiment; on the following days the T wave reached the same value as the R wave (Fig. 1, a) and isolated extrasystoles of different voltages and shapes were present. Injection of morphine on the 14th day caused bigeminy, but after the intravenous injection of calcium chloride, against a background of slow contractions (54 per minute),attacks of paroxysmal tachycardia appeared, lasting 10-15 seconds,which then changed into continuous paroxysmal ventricular tachycardia (see Fig. 1, a,b,c) . The attack of paroxysmal tachycardia continued for over 24 hours. On auscultation a systolic murmur and alternation were heard. On the 16th day the attack of paroxysmal ventricular tachycardia came to and end , leaving only solitary extrasystoles; T_3—high , trigeminy.

On the 17th day the T wave became low , and on the 31st day—negative, and T_2 remained high , with bigeminy. An increase in the tonus of the vagus center on the following days led to a slowing of the pulse rate, to the appearance of extrasystoles , to atrioventricular block (see Fig. 1, d) and to elevation of the T_2 wave. Increase in the tonus of the vagus center on the 42nd day caused atrioventricular block; the T_2 wave was considerably elevated. Ventricular fibrillation—sudden death of cardiac origin—supervened 25 minutes after injection of calcium chloride.

The heart at autopsy: in the anterior wall of the left ventricle there is a whitish , stretched scar, triangular in shape, measuring 4 × 2.5 × 2.5 cm; the wall of the left ventricle in the region of the scar is thinned and translucent.

The dog Volchok. Female, weight 15 kg, age 5 years. Very mobile, spirited. On April 21, 1958 the descending branch of the left coronary artery was ligated in its upper third. The dog was under observation in the

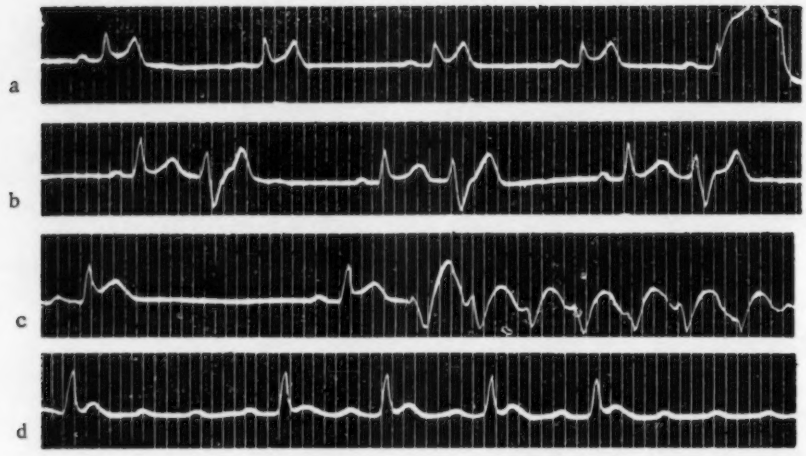

Fig. 1. Changes in the excitation of the heart with elevation of the tonus of the vagus nerve center in the dog Starik. a) Experiment on April 7, 1958, electrocardiogram (lead II) on the 14th day after ligation of the descending branch of the left coronary artery, before injection of morphine—monophasic curve; b) the same experiment, electrocardiogram (lead II) after injection of morphia—bigeminy; c) the same experiment, electrocardiogram (lead II) after the intraveous injection of calcium chloride—attack of paroxysmal ventricular tachycardia against a background of slow contractions of the heart; d) experiment on April 17, 1958, 24th day after ligation of the descending branch of the left coronary artery, electrocardiogram (lead II) after intravenous injection of calcium chloride—atrioventricular block.

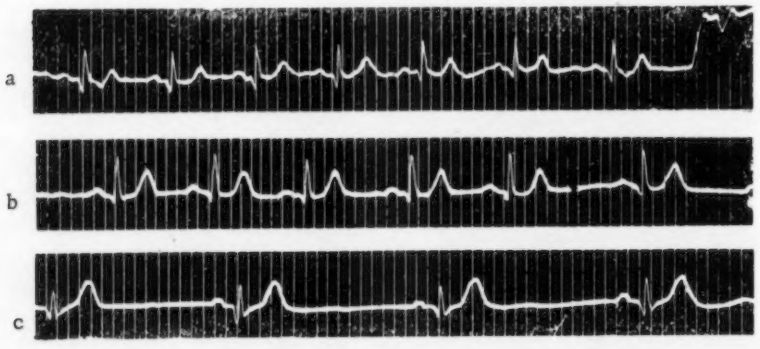

Fig. 2. Changes in the T_2 wave after elevation of the tonus of the vagus venter in the dog Volchok (16th day after ligation of the descending branch of the left coronary artery). a) Electrocardiogram (lead II) before injection of morphine, pulse 130 per minute; b) electrocardiogram (lead II) after injection od 2 ml of a 1 % solution of morphine, pulse 110 per minute; c) electrocardiogram (lead II) after injection (intravenously) of 10 ml of a 10 % solution of calcium chloride, pulse 76 per minute.

laboratory for 60 days. In the first 24 hours extrasystoles appeared, polytopic or in the form of bigeminy, and continued for 4 days. On the 5th day, after the tonus of the vagus center had been raised by injection of morphine, single left ventricular extrasystoles appeared; after the intravenous injection of calcium chloride considerable polytopic extrasystoles were observed, with elevation of the T wave, respiratory arrhythmia, slowing of the heart

rate from 100 to 56 per minute, increased heart sounds and a systolic murmur. On the 6th day the polytopic ventricular extrasystoles were still present. On the following days no extrasystoles were observed, Q_1 was very deep and T_3 below the isoelectric level.

On subsequent days (16th, 19th, 23rd, 25th, 29th) an increase in the tonus of the vagus center caused ventricular extrasystoles, sometimes polytopic and predominantly right ventricular, sometimes in the form of bigeminy, considerable elevation of the T_2 wave, respiratory arrhythmia and a slowing of the heart rate. The appearance of extrasystoles and elevation of the T_2 wave were observed after injection of morphine alone, but more especially after the intravenous injection of calcium chloride (Fig. 2). The T_2 wave reached the height of the R_2 wave, and sometimes even exceeded it. At the end of the experiment (after 1-1½ hours) the T_2 wave returned to its original magnitude. On the 36th, 43rd and 46th days, elecation of the tonus of the vagus nerve center led to the changes already mentioned and, in addition, atrioventricular block was observed after injection of calcium chloride.

On the 60th day the dog's general condition was satisfactory. On the electrocardiogram Q_1 was sharply pronounced and T_3 was below the isoelectric level. After injection of morphine the dog remained mobile and spirited, came into the laboratory by itself, jumped on to the bench and then, 2 minutes later, unexpectedly hung limp in its straps—sudden death of cardiac origin supervened (ventricular fibrillation).

The heart at autopsy: in the anterior wall of the left ventricle, below the site of the ligature, an aneurysm measuring 3 x 3 x 3 cm is seen; in this situation the wall of the left ventricle is grossly thinned, translucent and flabby; the thickness of the left ventricle is 2 cm, and of the right, 0.5 cm ; the lungs , liver and kidneys are congested.

The dog Lysyi. Male, weight 20 kg, age about 15 years (canine and incisor teeth sharpened, fur lacking in lustre , cornea cloudy). Relatively immobile, sluggish. On May 22, 1958 the descending branch of the left coronary artery was ligated in its upper third. The dog was kept under observation for 30 days. In the first few days after operation polytopic ventricular extrasystoles appeared and the heart rate was quickened to 120-140 per minute. On the 5th day, in order to raise the tonus of the vagus center, calcium chloride was injected intravenously against a background of the action of morphine . The initial state of the dog was satisfactory. The pulse rate was 108 per minute; solitary extrasystoles were present.

In the first minutes after the injection of calcium chloride the animal's condition deteriorated sharply : weakness of the action of the heart developed and the dog hung limp in its straps, showing signs of severe dyspnea; on auscultation the heart sounds were muted and there was a systolic murmur. On the electrocardiogram there was a significant increase in polytopic ventricular extrasystoles (mainly right ventricular), gross changes were present in the PQRST complex (lagging and ill-defined P wave, splitting of the apex of the R wave, T wave sometimes positive and sometimes negative, and so on). The elevation of the T_2 wave must be particularly mentioned. A gradual slowing of the heart rate (to 50 per minute) was observed 30-40 minutes after injection of calcium chloride. Parallel with this, the changes in the PQRST complex disappeared and the condition of the dog improved. On the 6th day no extrasystoles were observed and the pulse rate was 100 per minute; the general condition was satisfactory. On the 9th, 13th, 27th and 29th days after operation, elevation of the tonus of the vagus center led to the appearance of ventricular extrasystoles and to slowing of the cardiac contractions. Under these circumstances elevation of the T_2 wave was observed to appear occasionally after injection of morphine, and particularly of calcium chloride. The T_2 wave reached the height of the R_2 wave or even excceded it. In the acute experiment, on the 30th day stimulation of the peripheral cut end of the vagus nerve led to the appearance of single left ventricular extrasystoles against a background of the ordinary action of the vagus nerve. Extrasystoles were also observed after stimulation had ceased.

The heart at autopsy: below the site of the ligature there is a whitish scar measuring 5.5 x 2.5 cm, oval in shape; there are recent hemorrhages beneath the endocardium of the left ventricle; the thickness of the wall of the left ventricle is 1.5 cm, and of the right , 0.8 cm; the heart muscle is flabby.

The dog Rek. Male, weight 25 kg, age 6-8 years. Balanced. On May 29, 1958 the descending branch of the left coronary artery was ligated in its upper third. On the second day continuous polytopic ventricular extrasystoles appeared and the heart rate was quickened to 150-160 per minute. On the 6th day the extrasystoles gradually diminished and the heart rate reached its original value—70-80 per minute. On the 7th day the polytopic ventricular extrasystoles disappeared. Elevation of the tonus of the vagus center on the 7th and subsequent days did not cause extrasystoles, but led to slowing of the heart rate (to 52 per minute).

The young dogs tolerated experimental myocardial infarction quite well. The disturbance of cardiac activity was shown in these animals by the appearance of polytopic ventricular extrasystoles on the first or second day after operation, continuing usually for 5-6 days. During this time the vagus nerve did not exert its action on the heart in the form of slowing of the rate of contraction; elevation of the tonus of the vagus center led to intensification of the extrasystoles. Depending on the development of the collateral blood supply of the heart, its functional state gradually recovered, the extrasystoles disappeared and , on the 5th-6th day, the ordinary inhibitory influence of the vagus nerve on the heart reappeared. An example of this is the dog Rek , whose polytopic ventricular extrasystoles appeared on the second day after ligation of the descending branch of the left coronary artery, and lasted for 6 days . On the following days, elevation of the tonus of the vagus center caused merely a slowing of the heart's contractions. In the dog Donor, the extrasystoles lasted a little longer—8 days . On the 9th day, elevation of the tonus of the vagus center led to extrasystoles , and on the 11th day an attack of paroxysmal tachycardia was observed, which lasted about a week. On the following days, on raising the tonus of the vagus center, the usual action of the vagus nerve on the heart was observed. In the dogs Rek and Donor, thanks to the development of collateral coronary vessels, the cardiac activity was restored to normal.

It was shown in our laboratory that elevation of the tonus of the vagus center causes not only a slowing of the rate but also a strengthening of the contractions of the ventricles of the heart [7] . In consequence of this, during the development of myocardial infarction, disproportion may arise between the strength of the cardiac contractions and the blood supply of the myocardium, which considerably complicates the course of the infarct and even leads to sudden death of cardiac origin. This was clearly seen in the experiments on the dogs Volchok and Starik. Volchok was middle-aged and very mobile, and at first withstood the myocardial infarction with ease. As a result of repeated elevation of the tonus of the vagus center (10 times) the state of the cardiac activity was sharply altered. After elevation of the tonus of the vagus center on the 46th day, extrasystoles appeared and there was a considerable elevation of the T_2 wave. In subsequent experiments atrioventricular block took place. On the 60th day, elevation of the tonus of the vagus center, in conjunction with considerable physical effort (after receiving an injection of morphine, the dog ran, jumped on to the bench), led to sudden death—ventricular fibrillation supervened. In Starik , with atherosclerotic changes in the coronary vessels (age 12 years), as also in Volchok, after raising the tonus of the vagus center extrasystoles appeared, sometimes polytopic, sometimes in the form of bigeminy or trigeminy, together with atrioventricular block and elevation of the T_2 wave. In addition, attacks of paroxysmal ventricular tachycardia were observed, against a background of slow contractions. The tonus of the vagus center was raised 6 times in Starik; the last elevation, on the 42nd day, led to ventricular fibrillation.

The importance of the functional strain factor was confirmed also by the experiments with the old dog, Lysyi. This animal almost died from an attack of cardiac weakness when, on the 5th day after ligation of the descending branch of the left coronary artery, the tonus of the vagus center was elevated.

It is clear from these experiments that when the functional condition of the heart is altered (myocardial infarction), elevation of the tonus of the vagus nerve center in the dogs Volchok, Starik and Lysyi caused changes in the excitation of the specific musculature; this was manifest as a disorder of the cardiac action—as extrasystoles, paroxysmal ventricular tachycardia, atrioventricular block and ventricular fibrillation.

Stress must be laid on the trend of the T wave in Volchok, Starik and Lysyi: on raising the tonus of the vagus center, besides the disturbance of the cardiac rhythm, elevation of the T wave was observed, reaching the level of the R wave and sometimes exceeding it. This elevation of the T wave demonstrates the influence of elevation of the tonus of the vagus center on the restorative biochemical processes in the myocardium of the ventricles of the heart.

Repeated elevation of the tonus of the vagus center during experimental myocardial infarction, mainly in old dogs , may thus lead to complication of the course of the myocardial infarct by causing disturbances of the rhythmic activity of the heart and changes in the biochemical processes in the heart.

SUMMARY

Experimental myocardial infarction was induced in dogs of various ages. A rise of the vagus center tone was provoked by intravenous injection of calcium chloride solution against the background of morphine action at various periods following ligation of the descending branch of the left coronary artery. Notwithstanding their

briskness, cardiac arrhythmia is observed in the animals, especially old ones, for the period of several days after ligation. It manifests itself in the form of polytopic ventricular extrasystole, bigeminy, trigeminy, ventricular paroxysmal tachycardia or atrioventricular block, with even an occasional ventricular fibrillation, terminating in sudden death. With the rise of the vagus nerve center tone in dogs suffering from experimental myocardial infarction the biochemical processes of the ventricular myocardium become changed. This becomes clearly apparent in some dogs and manifests itself in considerable changes of the T wave which approaches the R wave in its size or even exceeds it.

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CHANGES IN THE ELECTROCARDIOGRAM AFTER BILATERAL ADRENALECTOMY IN DOGS

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It is well known from clinical observations and experimental research that the hormones of the adrenal glands affect many aspects of the activity of the body, including—and this to a very great degree—the cardiovascular system. Nevertheless the reports in the literature on the character and, especially, on the causes of the changes in the functional state of the heart in response to functional disturbances of the adrenal glands are contradictory.

The changes in the ECG in Addison's disease and in experimental interference with the function of the adrenal glands in animals have been described by several authors [3, 10, 13, 15, 17, 20, 22 and others]. Some of these [3, 9, 10, 12, 23] associate the changes in the heart's activity when adrenal function is disturbed with interference with the electrolyte balance (Na and K); other workers do not find this connection [14, 15, 21].

Other investigations have been made which indicate that the adrenal hormones affect the carbohydrate and protein metabolism of cardiac muscle. Removal of the adrenals leads to a considerable fall in the glycogen, ATP and creatine phosphate contents of the heart muscle. Administration of corticotonin (a physiologically active preparation obtained from the adrenal cortex by V. P. Komissarenko's method [4]) to an animal increases the glucose requirements of the muscle of the isolated heart and increases the content of energy-producing compounds in the cardiac muscle [5, 11]. These facts, it seems to us, do not support the assertion that the changes in the activity of the heart in adrenal insufficiency are due only to disturbances of the electrolyte metabolism.

We studied the activity of the heart (by the method of electrocardiography) in dogs after removal of the adrenals. By tracing the development of the ECG changes in adrenalectomized dogs, we tried to discover any possibility of compensation for these changes.

EXPERIMENTAL METHOD

Experiments were carried out on 9 dogs. After bilateral adrenalectomy (in two stages at intervals of 2 to 8 weeks) the animals were kept alive for a long period (from 2 to 20 months and even longer) by administration of desoxycorticosterone and saline solution, closely resembling plasma in its composition. As a result of this, the absence of adrenal cortical hormones regulating the water-salt metabolism was partially compensated, and the Na: K balance was not disturbed in these animals in a state of compensation. The animals received no glucocorticoids. In their outward appearance and behavior, these dogs were indistinguishable from normal.

In these animals which, thanks to the replacement therapy, were in a state of relative compensation, acute adrenal failure was caused by a change in the therapy. This was accompained by sluggishness, adynamia,

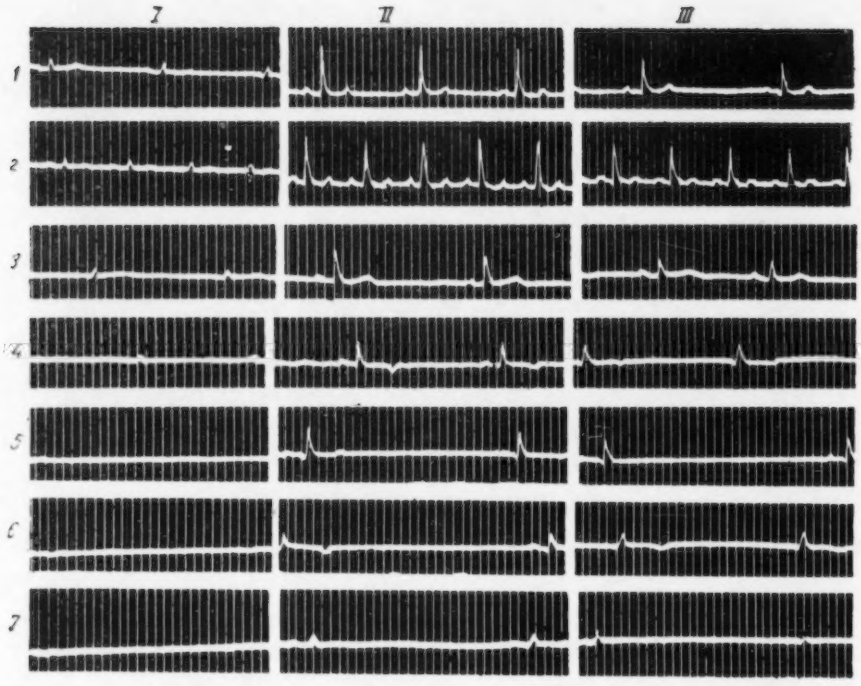

Fig. 1. Changes in the ECG of the dog Brovko after bilateral adrenalectomy. (In all the figures the ECG is shown for the 3 leads—I, II and III.) For explanation see text.

diarrhea, vomiting, a fall in the blood pressure, hemoconcentration, slowing of the bloof flow, an increase in the plasma potassium and loss of weight. Repetition of the course of replacement therapy brought the animal out of its crisis. Some animals endured several crises.

The ECG was studied before operation, after removal of one adrenal, after removal of the second and until the end of the animal's life. The changes in the ECG were studied in a state of compensation and during crisis. The ECG was recorded with the three classic leads, bythe usual method, by means of an EKP-4 apparatus.

EXPERIMENTAL RESULTS

The ECG was unchanged after the removal of one adrenal. The small variations which were due to the operation itself lasted one or two days. Removal of the second adrenal was accompanied in some animals by sharp, but brief, changes in the ECG mainly of the T wave: this was either greatly elevated or it became negative. In many animals marked tachycardia (up to 165-170 beats per minute) was observed in the course of one or two days after removal of the second adrenal.

Replacement therapy was given from the day of removal of the second adrenal, but, in spite of this, significant changes gradually developed in the ECG. In the first place the T wave and the S-T interval were affected. The change in the other components of the ECG took place much later. Very severe changes in the ECG were found only in the last days, and sometimes only in the last hours of the animal's life. Under these circumstances all the waves of the ECG were diminished, and especially the R wave (by 6-14 mm). The T wave was lowered and flattened; it often became negative and, in some cases, biphasic. In the last days of the animal's life the P wave, and sometimes the T wave also, disappeared. Thanks to the sharp fall in the main waves, the whole ECG at this time acquired an extremely flat and monotonous character. In the majority of the animals severe bradycardia developed (the heart rate did not exceed 50-40 per minute). The P-Q intervals were lengthened by 0.02- 0.06 seconds and the S-T intervals by 0.05-0.12 seconds. Replacement therapy did not prevent the changes in the ECG but merely retarded their development.

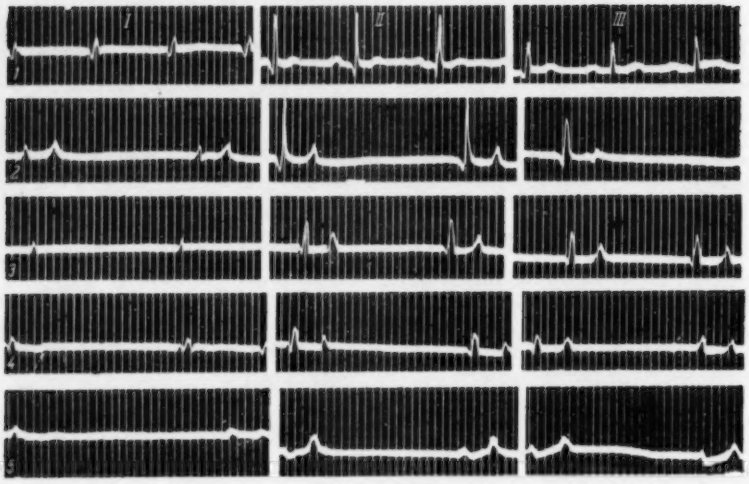

Fig. 2. Development of changes in the ECG during the last 4 days of life of the dog Pushok. For explanation see text.

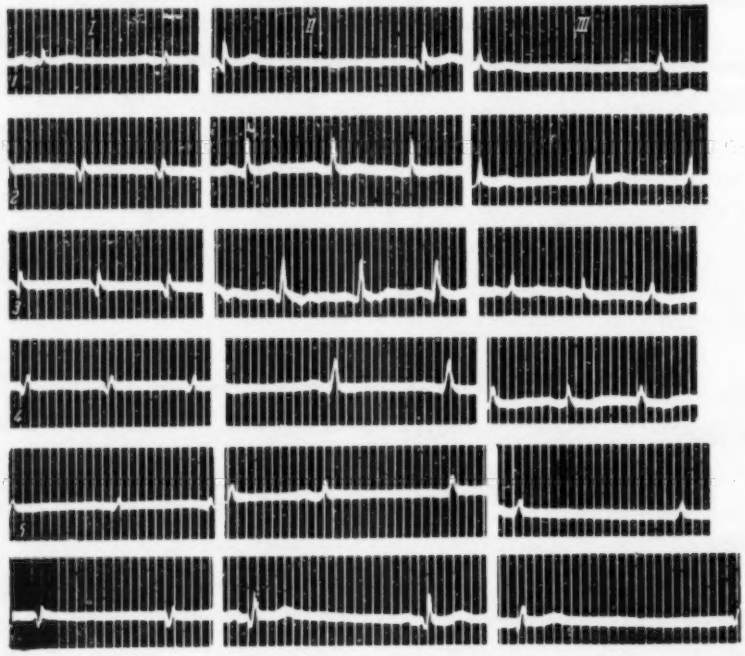

Fig. 3. Changes in the ECG in the period of development of and emergence from a repeated crisis in the dog Shalun. 1) ECG before discontinuation of replacement therapy; 2, 3, 4) ECG 5,7 and 10 days after discontinuation of replacement therapy; 5,6) ECG 3 and 8 days after resumption of the course of replacement therapy.

Fig. 1 illustrates the changes in the ECG after removal of the second adrenal from the dog Brovko. Cuts 1 and 2 show the ECG the day before (1) and the day after (2) operation; cut 3, the ECG 35 days after removal of the second adrenal (it kept this character throughout the whole time). Forty days after operation, 14 days

before the death of the animal, significant changes developed in the ECG (cut 4). During the next 10 days there was little change in the ECG (cut 6), and only in the 10-12 hours before the animal's death were the changes in the ECG greatly intensified (cut 7).

In Fig. 2 are shown the changes in the ECG in the last 4 days of life of the dog Pushok. During the 55 days after removal of the second adrenal, the character of of the ECG was almost unchanged (cut 1). Marked changes appeared only 3 days before the animal's death—the P wave disappeared, the character of the T wave was changed (cut 2). Subsequently the ECG changed every day (cuts 3, 4), and the changes reached their maximum degree 30-40 minutes before the animal's death (cut 5).

Both dogs received replacement therapy throughout this time.

When the animal's condition was good and the ECG kept its normal appearance, we temporarily ceased giving the saline solution and desoxycorticosterone, so that the role of the replacement therapy and the possibility of compensation for the changes in the ECG could be brought to light. Discontinuation of the replacement therapy led to severe and rapidly increasing changes in the ECG. On the 2nd-3rd day the heart rate rose to 150-170 per minute; on the 4th-5th day, still before the appearance of any external signs of crisis, changes occurred in the ECG (the T wave baceme negative and the S-T interval was shortened by 0.02-0.27 seconds); the ECG waves later diminished in size and they all gradually became smoothed out. On resumption of the replacement therapy the animal emerged from the crisis, although the changes in the ECG persisted for the first few days. The ECG regained its original form after 5-8 days.

Fig. 3 illustrates the characteristic changes in the ECG in the period of development of and emergence from a repeated crisis in the dog Shalun. Three days after discontinuation of the therapy, the heart rate rose from 60-68 to 100, and 6-7 days after, to 133-155 beats per minute. After 7 days, significant changes were observed in the ECG (cut 3); after 10 days these became more profound (cut 4). The animal's condition remained good during almost the whole of this time, and only 9 days after discontinuation of the therapy was slight sluggishness of its movement apparent. With the resumption of the course of replacement therapy, the changes in the ECG showed no weakening for one or two days (cut 5). The ECG regained its original appearance 8 days after the resumption of the therapy (cut 6).

During repeated crises the pattern of the changes in the ECG and of its return to its original appearance as the result of resumption of therapy was reproduced with remarkable accuracy. The only exception was the fact that after repeated crises the changes arose more rapidly and reached their maximun development in shorter periods of time than in the early crises. With resumption of the replacement therapy, the ECG regained its original appearance more slowly than after the previous crises. It seems to us that these factors indicate growing changes in the cardiac muscle after bilateral adrenalectomy, in spite of replacement therapy.

It must be pointed out that the rapidity of development and the depth of the changes in the ECG after bilateral adrenalectomy were not the same in the different dogs. Whereas some dogs lived a comparatively long time, maintaining a constant ECG in the period of compensation (Shalun — over 20 months), others died quite quickly (Kashtan lived 29 days, Orlik 14 days); under these circumstances the changes in their ECG in the day or two before death were either very weak or even absent altogether.

The results which we obtained testify to the high power of adaptation of the heart muscle to changes in the endocrine background of the utmost importance to the action of the heart. This is indicated by the maintenance of the constant character of the ECG for a long period of time in association with the profound disturbances of metabolism accompanying bilateral adrenalectomy. In this respect the results of our experiments agree with clinical observations, according to which, in the majority of cases of Addison's disease, significant changes are apparent in the last stages of the disease [9].

Our findings also suggest that the profound changes which develop eventually in the ECG of adrenalectomized dogs cannot be entirely explained by disturbances of mineral metabolism. The latter are compensated by the administration of saline and desoxycorticosterone. In a state of compensation the relative proportions of sodium and potassium in the serum were not disturbed. The possibility that relative compensation is, in fact, obtained with replacement therapy is shown by the sharp aggravation of the ECG changes when the therapy is discontinued and their disappearance soon after the therapy is resumed.

Replacement therapy did not, however, produce full compensation, for, although it was given for long periods of time, the dogs eventually developed significant ECG changes after bilateral adrenalectomy. These changes were evidently associated with interference with carbohydrate and protein metabolism, as a result of the absence of glucocorticoids. This is also confirmed by direct experiments [1, 2] showing that the protein and carbohydrate metabolism is considerably upset in adrenalectomized dogs on replacement therapy.

We have referred previously to the work of V. S. Lusenko [5], who showed that the content of glycogen, creatine phospahte and ATP in the heart muscle falls after adrenalectomy.

Finally, the character of the changes in the ECG after adrenalectomy may also serve as indirect evidence of changes in the metabolism of cardiac muscle. As we have pointed out above, the components of the ECG to be altered first after adrenalectomy were those which, in the opinion of many workers, are to some extent connected with metabolic processes, and according to A. I. Smirnov [7, 8], with restorative processes in heart muscle. This applies to the T wave, changes in which, in our experiments, appeared earlier and were more profound than in the other components of the ECG.

The association between the T wave of the ECG with metabolic processes has been shown by a number of special experiments in which authors have brought about changes in the carbohydrate and phosphorus metabolism of cardiac muscle and have observed changes mainly in the T wave [6, 8, 18, 19 and others].

Our results thus do not support the view that the profound changes which eventually develop in the ECG of adrenalectomized dogs are the result of interference with the mineral metabolism alone.

SUMMARY

The electrocardiographic changes developing in dogs following bilateral two-stage adrenalectomy were studied. The animal's life was maintained by desoxycorticosterone and saline solution administration. Changes appeared in the ECG only after the removal of the second adrenal gland, and were mainly revealed in the T wave and the S-T interval. In an animal in a compensated condition, these changes developed slowly and would become marked only during the last days, and, sometimes, even hours of its life. The ECG changes progressed more rapidly during the development of the crisis caused by the interruption of the replacement therapy. They could be revealed before the appearance of the external signs of the crisis.

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THE DETOXICATING ACTION OF ANTIBIOTICS OF THE TETRACYCLINE GROUP IN EXPERIMENTAL DYSENTERY TOXICOSIS

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Numerous investigations by clinicians have shown that antibiotics have a marked effect in diminishing the toxicosis in a number of infectious diseases. Penicillin is known to have an effective action in infections caused by cocci and chloramphenicol and the tetracyclines in typhoid and typhus fevers and dysentery.

The detoxicating action of antibiotics, and in particular of the tetracyclines, in experimental conditions has been studied less thoroughlythan the various aspects of their influence on microorganisms and on experimental bacterial infections [1-6, 9-11, 13].

There is as yet no single terminology to describe the effect of antibiotics on toxicoses. This effect is called "antitoxic", "antidotal", "disintoxicating" and, finally,"detoxicating". The mechanism of the detoxicating action of antibiotics continues to be uncertain.

The aim of the present investigation was to determine the characteristic features of the detoxicating action of the tetracyclines in experimental dysentery toxicosis.

EXPERIMENTAL METHOD

Toxicosis was induced by injecting white mice, weighing 16-18 g, with complete antigen prepared from Shigella flexneri by the method of tryptic digestion, at at the Moscow Mechnikov Institute of Vaccines and Sera.

The antigen was injected intramuscularly into the hindlimb in a volume of 0.1 ml of physiological saline or intraperitoneally in 0.25 ml. The MLD and the LD were determined for each antigen series and for the different methods of injection. As a rule, the MLD for intraperitoneal injection was $\frac{1}{2}$ to $\frac{1}{3}$ that for intramuscular injection. A single injection of 1 MLD of toxin led to the death of 80-100 % of animals in the course of 24-48 hours.

EXPERIMENTAL RESULTS

Histological examinations showed that in mice, as in certain other laboratory animals, dysentery endotoxin causes the severest changes in the spinal cord. The time of appearance and the degree of these changes depended on the method of injection and the dose of the toxin (see also [7, 8, 12]).

After the intramuscular injection of 1 MLD of toxin, the first changes in the motor neurones appeared after an average of 1 hour, and after intraperitoneal injection, in 30 minutes. The first phase, in which no changes were yet to be found, was thus of very short duration.

The second phase, lasting 4-8 hours, was characterized by a gradual development of reversible reactive changes. At first these consisted of extension of the tigroid bodies, an increase in the dimensions of the nucleoli

TABLE 1

Influence of Tetracyclines on the Course of Experimental Dysentery Toxicosis

Preparation	Method of administration	Dose of antibiotic (mg per mouse)	Number of mice in group	Number of mice dying
Chlortetracycline	Oxytetracycline	1.0	30	0
	Tetracycline	0.2	25	1
By mouth	Oxytetracycline	1.0	30	0
	Tetracycline	0.2	30	3
Intramuscularly	Oxytetracycline	1.0	30	0
	Tetracycline	0.2	20	2
Control	—	—	40	30

TABLE 2

Relationship between the Detoxicating Effect and the Time of Administration of the Antibiotic and the Dose of Toxin

Dose of antigen (mg per mouse)	Dose of tetracycline (mg per mouse)	Interval bet. adminis. of antigen and antibiotic	Number of mice in group	Number of animals dying
2,0	1,0	4 hours	15	0
2,0	1,0	6 hours	15	0
2,0	—	—	10	3
4,0	1,0	4 hours	14	2
4,0	—	—	20	8
6,0	1,0	simultaneuosly	20	3
6,0	—	—	20	11
8,0	1,0	simultaneously	20	3
8,0	—	—	20	20

and a rise in the content of desoxyribonucleic acid in the nucleus. Reactive division of the nucleoli took place subsequently. As the result of partial tigrolysis, the tigroid was arranged mainly around the nucleus (Fig. 1).

The third phase of the process was characterized by rapidly progressive degenerative changes in the motor neurones (pyknosis and hyperchromatosis of the nucleid, increasing tigrolysis, going as far as complete disappearance of the tigroid, and a sharp reduction in the volume of the cell body, Fig. 2). Degenerative changes of this type occurred on a large scala, especially in the lumbar and cervical divisions of the spinal cord. Changes in a large number of motor neurones led to the rapid development of paralysis, mainly of the hindlimbs. In animals dying 24-48 hours after injection of toxin, signs of neuronophagia were found. Similar, but less pronounced changes took place in the medulla oblongata of all the animals which died. Additionally, irrespective of the time of death, the animals showed constantly a massive hyperemia of the internal organs (Fig. 3) and multiple areas of extravasation. In roughly $\frac{1}{5}$ of the mice dying on the 2nd-3rd day, inflammatory lesions developed in the intestine.

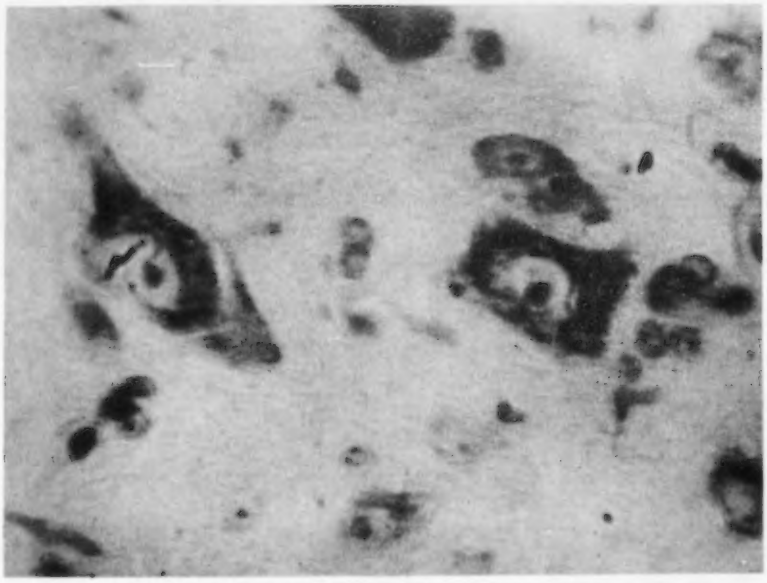

Fig. 1. Changes in the motor neurones of the lumbar division of the spinal cord of a mouse 6 hours after injection of 1 MLD of Flexner dysentery antigen. Tigrolysis of the neurone on the left, extension of the tigroid in the right neurone. Stained by Shabadash's method. Magnification 950×.

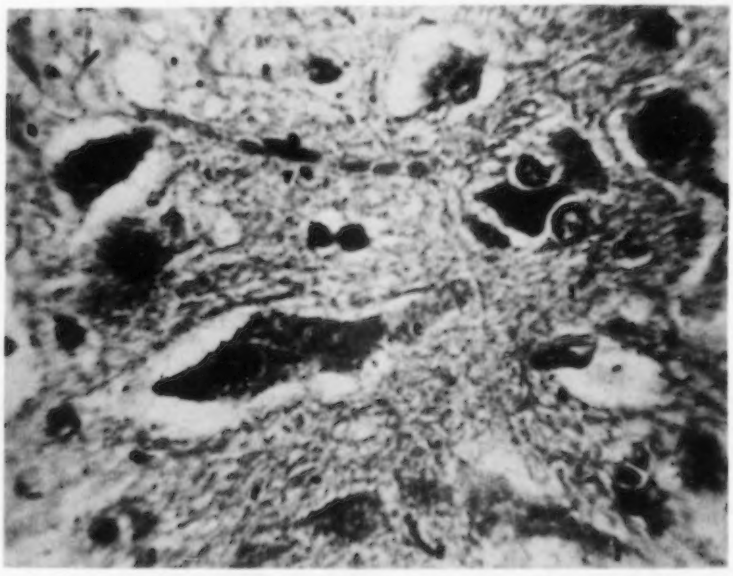

Fig. 2. Two deformed, degenerating motor neurones of the lumbar division of the spinal cord 24 hours after injection of 1 MLD of Flexner dysentery antigen. Magnification 1000×. Fixation by alcohol formalin, stained by Hansen's iron trioxyhematein method.

Antibiotics (chlortetracycline, oxytetracycline, tetracycline, penicillin and streptomycin) were given as a single dose either intramuscularly or by mouth, before or after injection of the antigen. Observations were continued for 7 days. At various times a number of control and experimental animals were sacrificed for the purpose of histological examination and blood culture on different media.

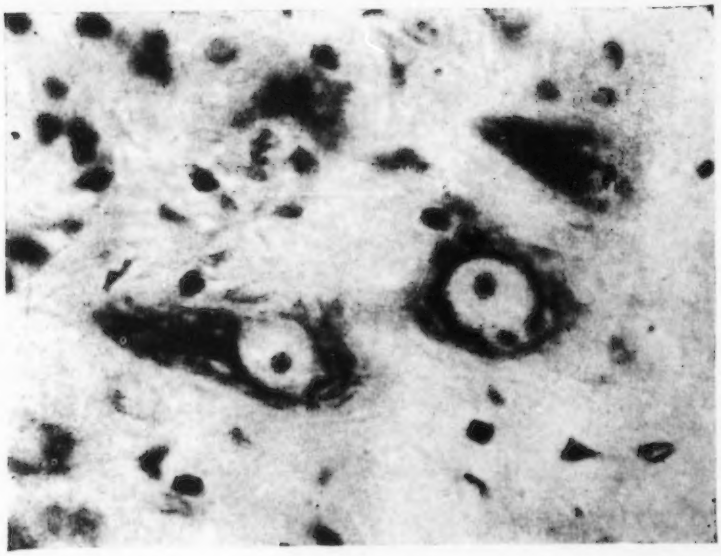

Fig. 3. Gross engorgement of a vein in the submucosal layer of the intestinal wall of a mouse during the development of dysentery toxicosis. Stained by Lepine's method. Magnification 600 x.

Using chlortetracycline as an example, it was shown that after intramuscular injection of 0.1 mg of antibiotic into a mouse in the course of one hour before injection of 1 MLD of toxin, the mortality among mice in the group receiving the antibiotic was 10%, whereas that among the control group of mice was over 80%.

This well-marked action of the tetracyclines on the course of the toxicosis was apparent when the antibiotic was given in the course of the hour, or immediately after injection of the antigen.

In Table 1 are shown the results of the action of the tetracyclines in toxicosis caused by the intramuscular injection of 1 MLD of whole antigen of Shigella flexneri. The antibiotics were given immediately after the antigen, by mouth or intramuscularly (Table 1).

As will be seen from the results in Table 1, both methods of treatment were effective: all the antibiotics prevented development of a fatal toxicosis in the animals. With oral administration all the animals survived, and when one fifth of this dose of antibiotic was injected intramuscularly, only solitary mice died.

In the majority of animals receiving antibiotics of the tetracycline group immediately after injection of dysentery toxin, degenerative changes (3 phases) were not present in the nerve cells. The reactive changes soon disappeared, and after 2-4 days were no longer found (the exceptions were those few cases in which the animals died in spite of administration of the antibiotic). Treatment with the tetracyclines prevented the development of any marked hemodynamic disturbances or of an inflammatory reaction in the intestine.

We have previously shown [1] that the administration of an antibiotic at later stages, for example 1 hour after the injection of 1 MLD of antigen, gives far inferior results and prevents the death of only a comparatively small proportion of animals.

Histological examination shed some light on the causes of this phenomenon. It was found that a detoxicating effect was possible if the antibiotic was given before the appearance of marked, morphologically detectable changes in the animals, and primarily in the central nervous system. The duration of this initial phase of the toxicosis determined the time interval during which the antibiotic was able to prevent death of the animals.

The shorter this first phase, the more quickly the antibiotic had to be given after injection of the toxin in order to show a detoxicating effect. Conversely, with a longer first phase, good results could be obtained with relatively late administration of the tetracyclines. Thus with an insignificant reduction in the dose of toxin, a detoxicating action is demonstrated when tetracyclines were given in the course of the first two hours,

and when the dose of toxin was halved, of the first 6 hours or more (Table 2). Meanwhile, in animals untreated with tetracyclines, even with half the dose of toxin, degenerative changes developed in time in the motor neurones of the spinal cord, although not on such a large scale as when 1 MLD was injected. The failure of the tetracycline series of antibiotics to show a detoxicating action in cases of far advanced toxicosis was due to the rapidly progressive and intensively expressed lesions in the more important systems of the body.

In identical conditions of dysentery toxicosis, penicillin, streptomycin and colimycin had no effect on the survival of the animals.

Concurrently with the histological examinations, at various phases of the toxicosis, blood from the animal's heart was cultured in meat-peptone broth and in Ploskirev's medium. In no case, as also in the healthy mice of the same batch, was bacteriemia discovered.

It was thus shown that chlortetracycline, oxytetracycline and tetracycline possess a marked detoxicating effect in experimental dysentery toxicosis of white mice. The degree of this detoxicating effect is dependent on the time and method of administration of the antibiotic. Under the same conditions other antibiotics — penicillin, streptomycin and colimycin — did not possess such an action.

We have shown [1] that the detoxicating effect of the tetracyclines is not due to neutralization of dysentery toxin as a result of direct fixation, and is only manifest in experiments in vivo.

The present investigation has shown that the tetracyclines prevent death of animals from toxicosis by preventing the development of severe degenerative changes in the central nervous system and other organs. Since this effect develops in the body in the absence of living microorganisms, there are no grounds for associating it with only the antibacterial action of the antibiotic.

Some authors suggest that the mechanism of detoxication is connected with the action of antibiotics on members of the so-called "normal" microflora, since toxicosis provides favorable conditions for proliferation of these organisms and for their penetration into the blood stream. Geller [6], on the basis of his observations, considers that this possibility is unlikely. In our own investigation, we did not find a single case of bacteriemia in mice into which antigen had been injected. In the conditions of these experiments the detoxicating effect could not, therefore, be due to the action of the antibiotics on the bacteriemia.

The establishment of the fact that a certain antibiotic has a detoxicating action makes a step forward in the explanation of the mechanism of its action in the body. The study of the detoxicating properties of antibiotics indicates new possibilities in the development of methods of rational treatment of those infections in which the toxicosis is of primary importance.

SUMMARY

Experiments were performed on white mice poisoned with the total antigen of Flexner dysentery bacilli. The detoxicating effect of chlortetracycline, oxytetracycline and tetracycline was demostrated both in oral and in intramuscular administration. A suitable dose of antibiotics prevented the degeneration of the cells of the central nervous system as well as the inflammatory changes in the intestines and the severe hemodynamic disturbances observed in the majority of untreated animals in dysentery intoxication. In treatment with tetracyclines the action is detoxicating manifested in the animals before the development of pronounced morphological changes in the central nervous system. This effect of tetracyclines is not caused by the neutralization of the toxin as a result of direct binding, nor by the action of the antibiotics on the bacteriemia (the latter, as shown by the authors' experiments does not developed in these conditions). Other antibiotics (penicillin, streptomycin and colimycin) had no detoxicating effect in analogous conditions.

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THE EXTERNAL SECRETORY FUNCTION OF THE PANCREAS IN EXPERIMENTAL DYSENTERY TOXICOSIS AND THE MECHANISM OF ITS DISTURBANCE

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It has now been established that the external secretory function of the pancreas is disturbed in patients with dysentery. Some authors [5, 13] account for the hypofunction of the pancreas under these conditions by the influence of depression of the secretory power of the gastric glands, others [3, 10] — by the influence of the diseased intestine, and a third group [2, 9, 11] — by the general effect of the dysentery toxicosis on the body. The weakened and perverted secretory reaction of the pancreas (to hydrochloric acid) is explained [4] by disturbance of the regulation of its activity by the central nervous system.

In view of the importance of the specific toxicosis in the pathogenesis of dysentery, we investigated in animals the influence of dysentery toxicosis on the external secretory function of the pancreas, and the mechanism of this influence.

EXPERIMENTAL METHOD

Experiments were carried out on dogs with a pancreatic fistula, created by A. N. Bakuradze's method [1].

It was possible, in these animals, to study the pancreatic secretion during eating (bread, meat, milk) and during injection of hydrochloric acid (30 ml of a 0.5% solution) into the duodenum. When not taking part in the experiments the animals did not lose their pancreatic juice. There were two variants of the experiments in which hydrochloric acid was injected. When one injection of acid was given, the pancreatic secretion was observed for one hour; when a sequence of several injections was given (every 30 minutes), observations were made for 4 hours.

In the course of the investigation estimations were made of the volume of pancreatic juice in ml during each hour and during the whole experiment, and also of its carbonate alkalinity (by a gasometric method in a Van Slyke apparatus), its amylase content (by our modification of the Goldstein-Michaelis method), the trypsin (by the Fuld — Gross — Michaelis method), lipase (by the method adopted at the Institute of Nutrition of the AMN SSSR) and the contents of organic and inorganic matter.

Dysentery toxicosis was induced in the dogs by the intravenous injection of toxin (0.15 mg of whole antigen of toxin from Hiss — Flexner dysentery bacteria per kg body weight). From 2 to 3 hours after receiving the injection of toxin, the animals developed the typical picture of dysentery toxicosis. In all, 128 experiments were performed on the two dogs Starik and Ledi over a long period of time.

TABLE 1

Changes in the Pancreatic Secretion during Eating before and after Induction of Experimental Dysentery Toxicosis

Indices of secretion	Initial background					2–4 days after injec. of toxin				
	Hours of secretion					Hours of secretion				
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
The Dog Starik										
Secretion in response to 300 ml milk										
Indices of secretion	24,5	13,0	19,5	10,5	9,0	65,5	39,0	15,5	12,6	14,1
Alkalinity (in volumes per cent)	105	67	90	105	50	250	240	200	135	140
Amylase content (in seconds)	330	375	210	195	210	720	600	480	330	285
Trypsin content (in conventional units)	1 280	640	1 280	1 280	2 560	320	320	320	640	640
Lipase content (in %)	14,5	14,5	17,4	37,8	37,8	8,7	14,5	17,4	20,3	20,3
Organic matter (in %)	1,85	1,91	1,86	3,86	1,69	1,17	1,09	1,51	1,85	1,35
Inorganic matter (in %)	0,77	0,83	0,76	0,70	0,94	0,86	0,93	1,02	0,86	0,84
Secretion in response to 125 g white bread										
Indices of secretion	51	37	26	19	12	84	61	40	34	26
Alkalinity (in volumes per cent)	150	110	55	60	55	215	252	210	162	130
Amylase content (in seconds)	345	280	255	180	105	765	840	675	510	495
Trypsin content (in conventional units)	2 560	2 560	2 560	2 560	2 560	640	640	640	640	640
Lipase content (in %)	11,6	11,6	11,6	17,4	23,2	5,8	0	5,8	17,4	20,3
Organic matter (in %)	2,09	1,97	1,76	1,14	1,72	0,98	0,89	1,87	1,47	1,88
Inorganic matter (in %)	0,85	1,00	0,45	0,99	0,45	1,00	0,95	0,93	0,80	0,77
The Dog Ledi										
Secretion in response to 50 g pounded meat										
Indices of secretion	10,5	23,0	20,0	17,0	6,0	29,0	26,5	26,0	22,5	22,0
Alkalinity (in volumes per cent)	60	105	105	95	50	155	140	130	120	120
Amylase content (in seconds)	270	210	180	135	90	915	945	910	750	645
Trypsin content (in conventional units)	1280	1280	1280	1280	1280	320	160	160	320	320
Lipase content (in %)	26,1	20,3	23,2	26,1	26,1	14,5	11,6	17,4	14,5	14,5
Organic matter (in %)	2,47	1,98	1,27	1,78	1,48	1,56	0,90	1,10	0,98	0,39
Inorganic matter (in %)	0,76	0,46	0,91	1,03	0,83	1,26	0,88	0,84	0,78	0,87

TABLE 2

Changes in the Pancreatic Secretion in Response to a Single Injection of Hydrochloric Acid

Time of investigation	Vol. of juice (in ml)	Carbonate alkalinity (in vol. per cent)	Amylase content (in seconds)	Trypsin content (in conventional units)	Lipase content (% decomposition of tributyrin)	Organic matter (in %)
The Dog Starik						
Initial background values	34,0	185	390	640	8,72	1,68
2nd day after injec. of toxin	42,4	200	502	640	5,81	1,13
The Dog Ledi						
Initial background values	24	157	645	640	8,72	—
2nd day after injec. of toxin	35	225	900	320	8,72	

TABLE 3

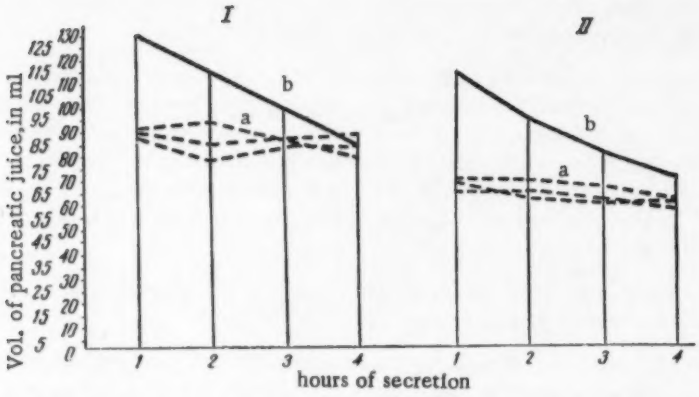
The Course of Recovery of the Pancreatic Secretion during Experimental Dysentery Toxicosis (secretion in response to 50 g meat)

	Initial background values	Days after injection of toxin						
		2nd	6th	11th	18th	36th	48th	64th
		The Dog Starik						
Volume of juice (in ml)	66,5—89,0 / 81	122,1	117,5	106,5	105,8	98	96	91
Alkalinity (in vol. per cent)	76—101 / 88	140,5	134	113	109,1	112	107	107
Amylase content (in seconds)	180—248 / 219	402	333	306	264	240	216	210
Trypsin content (in conventional units)	1280—2048 / 1536	898	1 024	1 352	1 664	1 536	1 780	2048
Lipase content (in %)	17,4—29,4 / 23,4	13,9	13,4	15,8	21,8	18,6	20,4	23,8
Organic matter (in %)	2,12—2,66 / 2,19	1,46	2,09	2,19	1,60		2,00	
Inorganic matter (in %)	0,44—0,97 / 0,74	0,6	0,79	0,84	0,98		0,70	

Note: In the colimn headed "Initial background values", the numerator gives the variations in the indices in 6 experiments and the denominator the mean value. The volume of juice is given as the total for the experiment; the other indices are the mean values for the hourly samples during the same 5 hours of the experiment.

EXPERIMENTAL RESULTS

It will be seen from the results in Table 1 that during experimental dysentery toxicosis, a sharp alteration took place in the typical course of pancreatic secretion in response to each of the food stimuli. The volume of



Changes in the volume of pancreatic juice during each hour of an experiment in which hydrochloric acid was introduced repeatedly (before and after injection of dysentery toxin).
1) In the dog Starik; II) in the dog Ledi . a) Determination of the background values (several experiments); b) on the 2nd day after injection of dysentery toxin.

pancreatic juice during each hour of working of the gland and throughout the whole experiment was increased. The alkalinity of the juice and the content of inorganic matter rose. The enzyme content (amylase , trypsin, lipase) and the organic matter decreased in amount. The pancreatic secretion evoked by injection of hydrochloric acid into the duodenum changed in the same direction (Table 2).

The changes were more pronounced in the first days after injection of the toxin. Gradually (in the course of about two months) recovery of the external secretory function of the pancreas took place (Table 3).

When the initial background values were studied in the experiment, it was found that the concentrations of enzymes and organic matter in the pancreatic juice secreted during eating were higher than those in response to injection of hydrochloric acid.

In the course of experimental dysentery toxicosis the changes in the quantitative and qualitative aspects of the secretory process were dissimilar. During eating, for instance, whereas the volume of the secretion was increased $1\frac{1}{2}$ -2 times, the enzyme concentration was lowered 3-11 times* . In response to injection of hydrochloric acid the increase in the volume of secretion was roughly the same, but the enzyme concentrations were lowered to a lesser degree or were quite unchanged. In the process of recovery of the secretory fuction of the pancreas, the volume of juice secreted recovered first and more completely.

These facts demonstrate that during experimental dysentery toxicosis, the enzyme-producing function of the pancreas was disturbed to a greater degree than the secretory function. In this connection it may be suggested that the nervous reflex mechanism was interfered with more than the humoral mechanism of regulation of the external secretory function of the pancreas.

As we know [8, 12] , the role of the humoral mechanism in the regulation of the pancreatic secretion is considerable. If the pancreatic secretion during dysentery toxicosis was increased during eating or administration of hydrochloric acid into the duodenum, then the cause of this phenomenon had to be sought in the mechanism of the secretory process caused by the introduction of hydrochloric acid into the duodenum. The hydrochloric acid of the gastric juice is known to play an important part in the combined working of the two organs.

Our investigations into the secretory function of the stomach in dogs with the same experimental dysentery toxicosis showed that the secretion of gastric juice and its acidity were lowered. The paradoxical increase in the pancreatic secretion may be connected with a change in the evacuatory power of the stomach (with accelerated

* According to the Borisov — Schütz rule, the concentrations of the enzymes are proportional to the squares of the values expressed.

entry of the acid gastric contents into the duodenum), or with a change in the functional capacity of the pancreas (with increased sensitivity of its neuroglandular apparatus to stimulation with hydrochloric acid).

These hypotheses were tested experimentally. For this purpose a series of experiments was carried out on two dogs with a Basov gastric fistula. It was found that the evacuatory power of the stomach in animals with experimental dysentery toxicosis was slowed. This slowing was shown especially in the first days after injection of the toxin, when the rate of the pancreatic secretion was increased maximally. For instance, whereas before injection of toxin, evacuation of 250 ml of a sour milk – starch mixture from the stomach was completed in 2 hours 45 minutes, after injection of dysentery toxin evacuation was completed in 4 hours 15 minutes, and after a further injection of toxin, in 5 hours 15 minutes.

The increased pancreatic secretion in dysentery toxicosis could not, therefore, be accounted for by changes in the secretory and evacuatory functions of the stomach.

In order to ascertain the functional state of the pancreas in experimental dysentery toxicosis, we carried out experiments in which pancreatic secretion was induced successively by repeated injections of hydrochloric acid into the duodenum for a long period of time. The results of some of the experiments in this series (see figure) show that whereas before injection of toxin the secretion after 4 hours of intensive working of the pancreas fell by 2.5-14.3% by comparison with that after one hour, on the second day after injection of the dysentery toxin it fell by 35.4-36.4%. With the course of time the functional capacity of the pancreas was restored.

The disturbance of the functional state of the pancreas in experimental dysentery toxicosis was thus demostrated as a paradoxical secretory reaction to hydrochloric acid and food products, and also as a more intensive exhaustion of the gland after prolonged activity. In turn, the disturbance of the functional capacity of the pancreas was evidently connected with changes in the adaptive and trophic influences of the nervous system.

There are indications in the literature of functional and organic changes in the different divisions of the nervous system in dysentery toxicosis [6, 7].

SUMMARY

Considering the significance of specific intoxication in dysentery pathogenesis, the author undertook the study of the external secretory function of the pancreas in conditions of experimental dysentery intoxication. Experiments were conducted on 2 dogs for a period of 2 months.

A considerable disturbance of the pancreatic response to food stimuli and hydrochloric acid was noted, manifesting itself by an increased rate of juice secretion and reduced concentration of the enzymes. Dysentery intoxication disturbs the mechanism of the nervous and humoral control of the gland external secretion and also deranges its functional condition. The glandular function is restored very slowly after administration of the toxin.

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BIOCHEMISTRY AND BIOPHYSICS

A COMPARATIVE STUDY OF THE PROTEIN FRACTIONS OF THE PLASMA AFTER A SINGLE EXPOSURE TO HIGH-ENERGY PROTONS AND TO X-RAYS

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In order to make a comparative study of the biological action of different forms of penetrating radiation, an investigation of the protein fractions of the plasma was carried out in rabbits exposed to the action of high-energy protons and in animals irradiated with x-rays.

Radiation sickness due to x-rays is accompanied by a very definite dysproteinemia, the characteristics of which was described in papers by several authors [1, 3, 4]. Information on the effect of protons of this order of energy is not be found in the literature.

EXPERIMENTAL METHOD

The study of the composition of the fractionated plasma proteins was carried out by the method of electrophoresis in a Tiselius-Svenson apparatus. Animals were exposed to the action of high-energy protons. The mean energy of the protons in the emitted beam was 480 Mev.

A layer of tissue of thickness 80 g/ cm^2 (80 cm) weakens the energy of 480 Mev protons to roughly 200 Mev. The value of the relative ionization in the tissue for this energy range of protons (200-480 Mev) remains practically constant (on the average 15 ion pairs/ micron of tissue), which is approximately the same as the relative ionization during exposure to x-rays with an energy of 200 kev.

The maximum ionization density (Bragg's curve) for primary protons with an energy of 480 Mev is situated at a distance of 110-120 cm [2]. Secondary processes, however (elastic and inelastic interaction of protons with nuclei of tissue elements) may bring about the appearance of particles with a higher relative ionization and, consequently, more effective in their biological action.

Animals in a securely fixed position were placed at a point in the first link of the proton tract at a distance of 5 and 45 cm from the outlet orifice, with the head directed toward the source of radiation. So that they could be compared with the action of x-rays, the doses of protons were expressed in physical roentgen equivalents. The measured dose rate on the body surface of one of the rabbits was 8.55 physical roentgen equivalents per minute.

In order to study the biological action of the high-energy protons, 17 rabbits were used, which received different doses of irradiation. The total dose of irradiation of the individual rabbits varied from 88 to 1220 physical roentgen equivalents. Blood was taken from the various rabbits for investigation, 1, 3, 9, 11, 19, 25, 31 and 50 days after a single exposure to different doses of protons. To compare the biological action of x-rays and protons, experiments were performed on 6 rabbits after exposure to equivalent doses of x-rays. The condictions of irradiation were as follows: voltage 180 kv, current 15 ma, filter 0.5 mm Cu + 1.0 mm Al, focal distance 60 cm, dose rate 18-25 r/ min.

Changes in the Protein Fractions of the Plasma of Rabbits Exposed to the Action of High-Energy (480 Mev) Protons and X-Rays.

Animal No.	Dose, rep	Time after exposure, in days	Protein fractions, in per cent				Fibrinogen
			albumins	globulins			
				α	β	γ	
	Normal (mean of 21 rabbits)		58,1±3,6	9,3±0,9	14,3±1,4	8,9±3,2	9,4±1,4
	Protons						
169	1 220	10	23,4	21,3	23,6	9,2	22,5
5	1 000	1	47,7	12,6	14,7	10,2	14,8
347	650	11	34,4	13,6	18,6	15,9	17,4
147	550	9	29,7	23,7	19,3	5,5	21,7
322	500	11	39,4	12,8	24,8	11,7	11,2
131	500	50	69,8	7,2	9,9	6,2	6,8
161	325	3	55,4	14,5	14,7	4,9	10,4
302	325	11	37,9	18,2	20,7	12,5	10,7
4	325	19	39,8	12,3	14,9	17,1	15,9
483	325	25	47,6	10,3	18,1	15,8	8,2
696	325	31	45,5	16,3	14,6	10,3	13,3
648	325	50	51,4	9,3	14,2	14,6	10,5
2	250	25	58,1	10,3	12,5	9,1	10,0
91	240	24	53,7	13,6	14,7	10,3	7,7
212	156	30	51,3	11,9	13,2	10,5	14,1
204	88	24	55,2	10,4	12,0	13,9	8,5
	x - Rays						
171	1 000	10	30,9	20,9	22,3	7,4	18,4
6	1 000	1	52,1	12,0	10,0	10,0	15,8
22	1 000	1	52,3	12,1	12,7	9,8	13,1
6	650	11	57,3	10,6	14,9	8,8	8,4
1	500	11	58,5	13,0	13,0	12,4	8,1
43	325	3	47,4	14,5	13,6	13,6	10,9
42	325	3	54,7	15,3	13,1	6,3	10,5
42	325	11	60,6	7,8	12,8	11,1	7,8
43	335	11	59,0	9,8	11,4	12,0	7,7
43	325	19	44,4	12,5	16,3	13,7	13,0
42	325	19	60,2	9,8	12,6	8,4	9,0
42	325	40	51,4	13,0	14,1	11,4	10,2

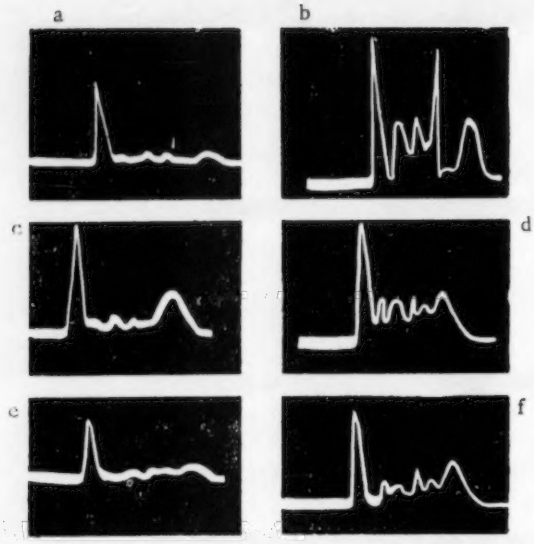
EXPERIMENTAL RESULTS

The results of the analyses for the two groups of animals are given in the table.

The results obtained show that as the disease developed, the relative proportion of albumins fell, the α- and β-globulin fractions and the plasma fibrinogen rose, while the changes in the γ-globulin fraction were not characteristic. The most considerable changes were found in animals irradiated with massive doses of protons.

In rabbit No. 169, for instance, on the 10th day after exposure to a dose of 1200 physical roentgen equivalents, a well-marked dysproteinemia was evidently found in the agonal period of the disease. An appreciable fall in the albumin fraction was detected in the first few days after exposure to protons in a dose of 1000 physical roentgen equivalents (see table).

Sublethal doses of protons (650, 550 and 500 physical roentgen equivalents) also caused well-marked variations in the composition of the plasma protein fractions. Analyses carried out on the 9th and 11th days after exposure to protons in the doses mentioned showed significant dysproteinemia (see figure, a and b).



Electrophoregrams of the plasma protein fractions of rabbits. a) Normal; b) 9 days after exposure to protons in a dose of 550 physical roentgen equivalents; c) 11 days after irradiation with x-rays in a dose of 325 physical roentgen equivalents; d) 11 days after exposure to protons in a dose of 325 physical roentgen equivalents; e) 19 days after irradiation with x-rays in a dose of 325 physical roentgen equivalents; f) 19 days after exposure to protons in a dose of 325 physical roentgen equivalents.

Comparison of the changes in the fractional composition of the plasma proteins, caused by x-rays and high-energy proteins in sublethal doses, at similar times after exposure, revealed a particularly great difference in the biological effect of these two types of radiation. Whereas on the 9th and 11th days after irradiation with protons, the rabbits showed considerable dysproteinemia, electrophoretic analysis of the plasma proteins at approximately the same time after x-rays irradiation did not show this feature.

With smaller doses (325 physical roentgen equivalents) no essential differences could be found in the actions of protons and x-rays in the first period of the disease (the first 24 hours). On the 11th and 19 th days, however, the difference was now apparent (see figure, c, d, e, f).

Electrophoretic analysis at a later period of the disease (on the 25th-31st day) showed some degree of normalization of the plasma protein relationships. On the 50th day full restoration to normal was observed, apart from a slight increase in the γ-globulin fraction (14.6 instead of a normal 8.9%).

Investigation of the protein fractions in rabbits exposed to the action of small doses of protons (from 88 to 250 physical roentgen equivalents) was carried out at a later period after exposure. In all the rabbits of this group that were examined the proportions of the plasma protein fractions remained within normal limits.

It must, therefore, be emphasized that dysproteinemia in animals exposed to the action of protons was more severe than in rabbtis irradiated with x-rays. Animals exposed to protons of such high energy values did, in fact, die after exposure to comparatively small doses (325-600 r), after showing signs of radiation sickness at relatively early stages.

If our findings are considered from the point of view of primary radiochemical processes, it may be postulated that the elastic and inelastic interaction of protons with the nuclei of the tissue elements (mainly carbon, nitrogen and oxygen) plays an essential role in the general effect of exposure of animals to radiation.

SUMMARY

By electrophoretic method with Tiselius apparatus the authors conducted a comparative study of the blood plasma protein fractions in rabbits following single irradiation with high energy protons and equivalent x-ray doses.

It was acertained that following irradiation with the protons, the fractional quantitative composition of blood plasma is altered in the same way as after x-ray irradiation: the albumin fraction level progressively decreases, while the α- and β-globulin fractions and fibrinogen contents rise. The changes consequent upon proton action, however, are more intense, this, evidently, resulting from the formation in the tissues of secondary ionized particles, more effective biologically than the protons.

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THE EFFECT OF D,L-CYCLOSERINE ON THE PROCESS OF TRANSAMINATION

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According to the findings of Yamade, Sawaki and Hayami [5], the new antituberculous drug D-cycloserine suppresses the decarboxylation and decomposition of tryptophan in cell-free preparations of bacterial enzymes in lower concentrations than does isoniazid. The addition of pyridoxal or phosphopyridoxal restores the suppressed processes.

These authors also showed that cycloserine forms complexes with vitamin B_6.

N. K. Kochetkov and his co-workers suggest that the antibacterial action of cycloserine is based on disturbance of nitrogen metabolism as the result of the formation of azomethine compounds of cycloserine with the pyridoxal coenzyme. In this connection it appeared to be of interest to study the influence of both cycloserine itself and of other compounds with analogous functional groups on the process of transamination.

According to A. E. Braunshtein and M. M. Shemyakin [1], the process of transamination begins by the formation of an azomethine complex of phosphopyridoxal-protein with an amino acid. It is, therefore, quite possible that the distinctive amino acid cycloserine plays the role of an antimetabolite in the transamination reaction.

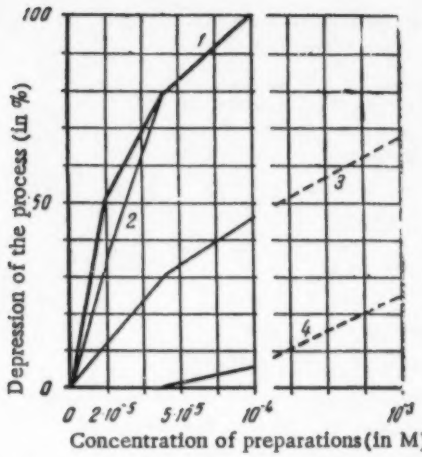
In the present research we studied the effect on transamination of D,L-cycloserine and of three other compounds obtained by N. K. Kochetkov and his co-workers: cyclothreonine (preparation KKh-11), the dihydrochloride of the ethyl ester of β-aminooxyalanine (preparation KKh-9) and the semihydrochloride of β-aminooxyalanine (preparation KKh-14). The effect of these compounds on the process of transamination was investigated in rat liver homogenates, incubated with glutaminic and pyruvic acids.

EXPERIMENTAL METHOD

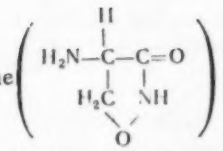
White rats, weighing 100-150 g, were decapitated and the liver was rapidly extracted and homogenized in the cold, in a glass homogenizer, with a phosphate buffer at pH = 7.4. Composition of the samples: 0.7 ml phosphate buffer, 0.1 ml of a 0.2 M solution of glutaminic acid, 0.1 ml of a 0.2 M solution of pyruvic acid and 0.1 ml of 20 % homogenate.

In a control sample, the pyruvic acid was replaced by buffer. Inhibitor solutions of various concentrations were added in volumes of 0.1 ml instead of buffer. The solutions of glutaminic acid and pyruvic acid, and also the inhibitor solutions, were preliminarily neutralized with NaOH.

The samples were incubated at 37° for 1 hour and then fixed by the addition of 1 ml of 20% trichloroacetic acid. The latter was removed from the filtrates by extraction five times with ether; after removal of all traces of ether, the amino acids were separated by the method of ascending chromatography on paper, in a mixture of butanol, acetic acid and water (in proportions of 4:4:1). Alanine and glutaminic acid were estimated

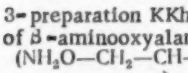


The effect of D,L-cycloserine and preparations KKh-9, KKh-14 and KKh-11 on transamination in rat liver homogenates.

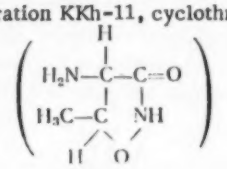
1—D,L-cycloserine  ;

2- preparation KKh-9, the dihydrochloride of the ethyl ester of β-aminooxyalanine

3- preparation KKh-14, the semihydrochloride of β-aminooxyalanine



4- preparation KKh-11, cyclothreonine



quantitatively by the method of Giri and Rao [3]. The intensity of the process was estimated by the decrease in the glutaminic acid content and the increase in that of alanine.

The R_f was determined for all compounds capable of being developed by ninhydrin. Cycloserine and preparation KKh-9, in the same concentrations as glutaminic acid, produced lightly stained, small spots with and R_f equal to that of glutaminic acid. Since cycloserine and preparation KKh-9 were used in the experiments in considerably lower concentrations than glutaminic acid, we considered it possible to disregard superimposition of these spots. The spots of preparations KKh-14 and KKh-11, also lightly stained, were almost completely merged with the alanine spot.

In some cases these preparations were used in concentrations only 10 times lower than the concentration of the substrate, but in these cases, too, the results obtained in respect to decrease in the glutaminic acid content did not differ appreciably from those obtained in respect to the increase in alanine.

EXPERIMENTAL RESULTS

The experimental results (see figure) show that, starting at concentrations of $2 \cdot 10^{-5}$ M, cycloserine caused considerable depression of the process of transamination A similar action was shown by the preparation KKh-9. Differences in the actions of this compound and cycloserine could be observed only at very low concentrations. The preparation KKh-14 depressed transamination much more weakly. The least effect on the transamination process was shown by preparation KKh-11.

The high activity of preparation KKh-9 was probably explained by the fact that this compound very readily underwent a cyclic change, and at a pH of 7.4 was converted into cycloserine [2]. The possibility of the direct action of this preparation itself cannot, however, be excluded.

The direct conversion of preparation KKh-14 into cycloserine in mild conditions is impossible [2], and at the same time it inhibited transamination more weakly, but, nevertheless perceptibly.

It may be suggested that the cyclic structure and the unsubstituted hydrogen in position 5 of the cycloserine molecule had some connection with the stabilization of its azomethine compound with pyridoxal or with phosphopyridoxal, since the presence of a free amino group in the preparations KKh-14 and KKh-11 enabled the formation of azomethine compounds, and the effect of these

* It was shown by special experiments that on replacing glutaminic acid by ammonium carbonate, only negligible amounts of alanine were formed, and this was unaffected by cycloserine. We consider, therefore, that when liver homogenates were incubated with glutaminic and pyruvic acids, cycloserine depressed the formation of alanine by the transamination reaction but not by direct amination.

The Effect of Pyridoxal and Phosphopyridoxal on the Restoration of the Process of Transamination when Suppressed by D,L-Cycloserine

Test No.	Added to the complete test	Decrease in glutaminic acid content, in mM / g tissue	Increase in alanine content per g tissue
1	—	0,65	0,50
2	$5 \cdot 10^{-5}$ M D,L-cycloserine	0,13	0,10
3	$5 \cdot 10^{-5}$ M D,L-cycloserine + $5 \cdot 10^{-4}$ M pyridoxal	0,10	0,01
4	$5 \cdot 10^{-5}$ M D,L-cycloserine + $5 \cdot 10^{-4}$ M pyridoxal + 10^{-4} M ATP	0,15	0,01
1	—	0,70	0,60
2	$5 \cdot 10^{-5}$ M D,L-cycloserine	0,20	0,12
3	$5 \cdot 10^{-5}$ M D,L-cycloserine + $2,5 \cdot 10^{-4}$ M phosphopyridoxal	0,20	0,10
1	—	0,70	0,54
2	$2 \cdot 10^{-5}$ M D,L-cycloserine	0,34	0,20
3	$5 \cdot 10^{-5}$ M D,L-cycloserine + 10^{-4} M phosphopyridoxal	0,25	0,20

By analogy with the action of cycloserine on the process of decarboxylation it may be suggested that pyridoxal or phosphopyridoxal will also restore the process of transamination, when suppressed by this antibiotic.

To test this hypothesis we carried out experiments in the same conditions as before*. Pyridoxal or phosphopyridoxal were added to the mixture buffer, substrate, cycloserine, and 10-15 minutes later the homogenate was added.

As will be seen from the experimental results shown in the table, neither pyridoxal, alone or with ATP, nor phosphopyridoxal restored the process. It was evident that the azomethine compound of cycloserine with free pyridoxal and phosphopyridoxal was unstable in the absence of a breakdown enzyme. If this were not so, pyridoxal or phosphopyridoxal, when added to cycloserine before the addition of the hemogenate, would be bound to neutralize it. It may thus be postulated that a stable complex is formed as a result of the combination of phosphopyridoxal-protein with cycloserine, and that the protein is of importance in the stabilization of the azomethine compound of cycloserine with phosphopyridoxal.

Hicks and Cymerman-Craig [4] came to a similar conclusion in respect to other transamination poisons — the hydrazides. They consider that hydrazides are combined, not with free phosphopyridoxal, but with phosphopyridoxal combined with protein. From this point of view, no explanation can be given of the findings of Yamade and his co-authors [5], who not only observed restoration of the processes of decarboxylation and decomposition of tryptophan by free pyridoxal or phosphopyridoxal, but also, by means of the absorption spectrum and by separation by means of paper chromatography, showed that a cycloserine — pyridoxal complex was formed.

This problem, as also that of the influence of cycloserine on the other aminoferases, is at present being investigated by us.

SUMMARY

The authors studied the effect of D,L-cycloserine, the dihydrochloride of the ethyl ether of β-aminooxyalanine (preparation KKh-9), semihydrochloride of β-aminooxyalanine (preparation KKh-14) and of cyclothreonine (preparation KKh-11) on the amino group shift from glutaminic acid to pyruvic acid in rat liver homogenates.

* Phosphopyridoxal was kindly made available to us by Prof. A. E. Braunshtein to whom we extend our deep gratitude.

D,L-cycloserine and preparation KKh-9 in 10^{-5}M to 10^{-4}M concentration, tend to depress this process by 50-100 %. Preparation KKh-14 depresses the process by 45% in 10^{-4}M concentration and by 70% in 10^{-3}M concentration. 10^{-3}M concentration of preparation KKh-11 depresses the process by 25% only.

An admixture of pyridoxal or phosphopyridoxal, in concentration 5 times above that of D,L-cycloserine, did not restore the process depressed by the latter.

It is conjectured that as a result of the combination of phosphopyridoxal-proteid with cycloserine a stable complex compound is formed; for the stabilization of the above complex the cyclic structure and the non-substituted hydrogen of the cycloserine molecule in the 5th position is important.

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THE EFFECT OF GLUTAMINIC ACID ON CERTAIN METABOLIC PROCESSES IN ANOXIA AND DURING PHYSICAL WORK

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We have shown in previous papers [2, 3] that the administration of glutaminic acid to animals in anoxia stimulates their oxygen demand. The hypothesis was put forward that this effect, in these conditions, is dependent on the more intensive inclusion of incompletely oxidized metabolic products in the oxidation cycle of the three-carbon acids.

In order to test the reliability of this hypothesis, we investigated the effect of glutaminic acid on the content of incompletely oxidized metabolic products in the blood (oxygen debt), on the blood sugar and lactic acid contents and on the lactic acid and glycogen contents of the liver. The adenosinetriphosphate (ATP) content of the liver, skeletal muscle and brain was also determined. Experiments were carried out on animals kept in a gaseous environment deficient in oxygen. Under these circumstances an accumulation of incompletely oxidized metabolic products and of lactic acid is known to be observed in the body, and there is a considerable utilization of the glycogen reserves and of high-energy compounds [6-11, 13, 14, 16].

EXPERIMENTAL METHOD

Experiments were performed on adult white rats. In order to ensure approximately equal glycogen contents in the liver and muscles of the experimental animals, the rats used were preliminarily starved for 24-30 hours, and were given subcutaneous injections of glucose, in a dose of 5 mg/ g body weight, 1½ hours before the experiment. One of the animals (experimental) was injected 30 minutes later with sodium glutamate (1 mg/ g body weight), and both rats were then kept for 2 hours in a specially constructed apparatus ensuring a stable oxygen content of the inspired gaseous mixture (10-12 %) and absorption of the expired carbon dioxide. The animals were then quickly decapitated; after exsanguination, their tissues were frozen in liquid nitrogen and subjected to biochemical investigation. In this series 13 experiments were carried out on 26 animals. The results obtained were subjected to statistical analysis.

EXPERIMENTAL RESULTS

According to the results obtained (Table 1), the administration of sodium glutamate to animals kept in a lowered partial pressure of oxygen led to a considerable decrease in the level of incompletely oxidized products (by 36.3 %) and of lactic acid (by 30.3 %) in the blood, by comparison with their concentration in control animals. There was also an appreciable fall in the lactic acid content of the muscles (by 23.2 %). So far as the glycogen content of the tissues was concerned, this was only slightly changed in the skeletal muscles and remained at a high level in the liver. In the cardiac muscle of the experimental animals the glycogen level was also much higher than in the control animals (by 52 %). The ATP content of the experimental animals was higher than that of the controls, especially in the brain tissue (by 61 %).

TABLE 2

The Effect od Administration of Glutaminic Acid on the Metabolic Indices during Swimming (mean values, in mg%, of 20 experiments)

Indices	Swimming for 15 minutes			Swimming for 1 hour		
	Control	Experiment	difference, %	Control	Experiment	difference, %
BLOOD						
Sugar	112±3,4	129±2,3	+15	106,2±5,12	121,7±5,3	—15
Incompletely oxidized products (oxygen debt)	123,2±5,96	99,5±5,38	—19	147±4,97	109±3,3	—25
Lactic acid	75,0±1,3	59,0±1,38	—21	61,4±2,7	49,6±1,6	—19
SKELETAL MUSCLE						
Glycogen	394±15,6	479±9,3	+21	307±11,4	372±12	+23
Lactic acid	230,4±9,9	174,6±3,89	—24	191,7±4,06	165,5±6,35	—16
LIVER						
Glycogen	1115±78,6	1178±77,6	+6	794±42,9	861±32,5	+8

Note: Apart from the liver glycogen, the changes in the other indices were statistically significant.

It will be seen from the results in Table 2 that, in the rats receiving preliminary injections of sodium glutamate, after 15 minutes' swimming the content of incompletely oxidized products in the blood was less than in the control animals by 19.3%, and after swimming for 1 hour by 25.4%; the lactic acid concentrations were 21.4 % and 19.3 % less respectively. The blood sugar levels of the experimental animals were also slightly higher.

A lower lactic acid concentration was also found in the skeletal muscles of the experimental animals: after swimming for 15 minutes by 24.2 % and after 1 hour by 16%. The glycogen content of the muscles and liver of the rats receiving sodium glutamate remained at a higher level: in the muscles after swimming for 15 minutes by 21%, after one hour by 23%; in spite of the fact that the difference between the values of the glycogen content of the liver of the experimental and control rats was not statistically significant, a tendency was shown for the glycogen content in the experimental animals to remain at a higher level.

The results of these experiments thus show that during intensive physical work, the preliminary injection of sodium glutamate brings about better utilization of intermediate products of metabolism and maintenance of the carbohydrate reserves of the animal. These findings were, in fact, similar to those which we obtained in the anoxia experiments.

As long ago as in 1936 it was shown [18] that glutaminic acid supports the respiration of brain sections, suppreses anaerobic glycolysis [19] and maintains aerobic glycolysis. This is associated with the fact that glutaminic acid also plays an important role in the oxidation cycle of the three-carbon acids [1].

In experiments on the mitochondria of the heart [17], it was shown that each member of the cycle readily oxidizes pyruvate. The work of M. F. Gulyi et al. [5] has shown that administration of one of the components of the cycle — citric acid — to rabbits with alloxan diabetes and to human patients with glycosuria lowers the hyperglycemia and the glycosuria, the ketonuria and the polyuria. It has further been shown that, during fatiguing work, citric acid leads to smaller changes in the metabolism of purines and ATP of the working muscle of the rabbit, and also halts the excretion of purines in the urine in man [4]. These findings demonstrate that when oxidative processes are diminished in the body, they may to some extent be restored by administration of citric acid.

It can be seen from our experiments that glutaminic acid also possesses an analogous action and that during intensive physical work, disturbances also take place in the three-carbon acid cycle which may,

During anoxia , therefore, sodium glutamate undoubtedly brought about a more intensive inclusion of incompletely oxidized products of metabolism in the aerobic phase of oxidation, thereby securing a more economical utilization of the glycogen reserves and of high-energy compounds.

In connection with these findings, we thought it of interest to investigate the effect of sodium glutamate on the concentrations of the above -mentioned constituents of the blood and tissues during intensive physical work, and in particular in rats during swimming. According to reports in the literature [12, 15], under these circumstances a considerable increase takes place in the lactic acid content of the blood and muscles, with a fall in the glycogen content of the muscle tissue, i.e., biochemical changes closely resembling those observed during anoxia.

TABLE 1

The Effect of Administration of Glutaminic Acid on the Metabolic Indices during Anoxia (mean values, in mg %, of 13 experiments)

Indices	Control	Experiment	Difference, in %	Statistical oxygen significance
BLOOD				
Sugar	93,5±2,79	107±2,79	+14	Significant
Incompletely oxidized products (oxygen debt)	152±7,6	107,2±8,44	—36,3	»
Lactic acid	38,0±2,2	26,5±1,85	—30,3	»
SKELETAL MUSCLES				
Glycogen	474,5±20	504,6±17,5	+6%	Not significant
Lactic acid	169,4±9,75	130,1±8,7	—23,2	Significant
ATP	29,0±1,42	32,0±1,37	+10,2	Not significant
LIVER				
Glycogen	1097±66	1367±65	+24,6	Significant
ATP	12,3±0,19	15,9±0,19	+29	»
BRAIN				
ATP	15,4±1,26	22,2±1,58	+61	»
HEART MUSCLE				
Glycogen	544±25,4	808±29	+52	»

During anoxia, therefore, sodium glutamate undoubtedly brought about a more intensive inclusion of incompletely oxidized products of metabolism in the aerobic phase of oxidation, thereby securing a more economical utilization of the glycogen reserves and of high-energy compounds.

In connection with these findings, we thought it of interest to investigate the effect of sodium glutamate on the concentrations of the above-mentioned constituents of the blood and tissues during intensive physical work, and in particular in rats during swimming. According to reports in the literature [12, 15], under these circumstances a considerable increase takes place in the lactic acid content of the blood and muscles, with a fall in the glycogen content of the muscle tissue, i.e., biochemical changes closely resembling those observed during anoxia.

Experiments were carried out on adult white rats of the same sex and weight. As in the previous series of investigations, 4 animals were starved for 24-30 hours and then injected subsutaneously with glucose solution (5 mg/ g body weight). Sodium glutamate was injected 30 minutes later (1 mg/ g body weight) into 2 experimental rats, and the rats were placed after one hour in water at a temperature of 35-36°. After swimming for 15 minutes the rats were sacrificed. In another series of experiments the animals were sacrificed after swimming for one hour. In all, 20 experiments were performed. The results were subjected to statistical analysis.

evidently, be compensated by the administration of this amino acids. The concrete mechanism of this effect requires further investigation.

The higher glycogen content in the liver and heart muscle and ATP content of the tissue of the brain of the animals receiving glutaminic acid, which we observed in our experiments, emphasizes yet again the power of this amino acid to normalize the metabolism, by virtue of the more intensive inclusion of incompletely oxidized products of metabolism in the aerobic phase of oxidation. This action of glutaminic acid is shown particularly clearly in tissues which are most sensitive to oxygen deficiency (brain, heart muscle and liver).

SUMMARY

The effect of administration of glutaminic acid on the content of incompletely oxidized metabolic products (oxygen debt), lactic acid, glycogen and adenosinetriphosphate was studied in anoxia, as well as after physical exertion (swimming). It was established that glutaminic acid promotes the reduction of the blood level of incompletely oxidized metabolites and of lactic acid, decreases their accumulation in the muscles, maintains the glycogen content of the liver and especially of the cardiac muscle, as well as the concentration of adenosinetriphosphate in the brain at a higher level. In experiments with swimming it was demonstrated that preliminary administration of glutaminic acid promotes a lesser accumulation of incompletely oxidized products and lactic acid in the blood and decreases the glycogen utilization in the muscles. A suggestion is made that this effect is due to stimulation of the oxidative processes by glutaminic acid at the expense of more intense involvement of the incompletely oxidized metabolites in the tricarboxylic cycle.

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THE BLOOD SEROTONIN CONCENTRATION IN MONKEYS

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The isolation of serotonin (5-oxytryptamine) in pure form from ox serum by Rapport, Green and Page in 1948 [11, 12] and its subsequent synthesis in a number of laboratories [7, 13] formed the beginning of a large number of investigations to study the biological properties of this amine in the healthy animal and in several pathological states.

The results of these investigations, summarized in numerous surveys [1, 6, 9, 10], show that serotonin or 5-oxytryptamine possesses important pharmacological, physiological and biochemical properties, which justify its being classified among such biologically active compounds as adrenalin, histamine and acetylcholine.

Of great importance in the elucidation of the physiological role of 5-oxytryptamine is the quantitative data concerning the distribution of serotonin among the animal world. The concentration of 5-oxytryptamine in the blood of different species of vertebrate and invertebrate animals has been investigated in some detail [5, 6, 8, 14].

Among the abundant literature on this subject, however, we could find no work on estimation of the concentration of 5-oxytryptamine in the blood of monkeys, and the present research was accordingly conducted with this object.

EXPERIMENTAL METHOD

The investigations were conducted at the Sukhumi medicobiological station in April — May 1958. Experiments were carried out on monkeys of the Macacus rhesus and Papio hamadryas species.

Blood for determination of the 5-oxytryptamine was taken from the cubital vein of the monkeys, in a volume of 3-5 ml. To prevent clotting, a 3.8 % solution of sodium citrate was added, in a dose of 0.1 ml per ml of blood.

The 5-oxytryptamine was extracted from the blood with acetone for a period of 24 hours at a temperature of 4 to 5°. After the precipitate had been removed by filtration, the acetone was evaporated from the samples in vacuo at 35° C. The dry residue was dissolved in Gaddum's fluid, which was also used for nutrition of the isolated gut. The quantitative determination of the 5-oxytryptamine was carried out on the isolated colon of a rat by the method of Dalgleish et al. [2]. The standard used was 5-oxytryptamine creatinephosphate, made by the firm Fluka (Italy).

EXPERIMENTAL RESULTS

The results of the estimations of 5-oxytryptamine in the whole blood of monkeys are shown in Table 1. As will be seen from the figures in Table 1, the concentration of 5-oxytryptamine in the blood of monkeys showed considerable fluctuation, which could be dependent on the lability of the composition of the peripheral

TABLE 1

Serial No.	Species	Sex	Serotonin concentration, γ/ml
1	Macacus rhesus	Male	1,6
2	»	»	0,5
3	»	»	0,32
4	»	»	1,12
5	»	»	1,6
6	»	»	1,28
7	»	»	0,66
8	»	Female	0,01
9	»	»	0,12
10	»	»	0,62
11	»	Male	0,49
12	»	»	0,99
13	»	»	0,06
14	»	»	0,59
15	»	Female	0,81
		Mean	0,70±0,10
1	Hamadryas	Male	1,32
2	»	Female	0,33
3	»	"	2,00
4	»	Male	0,42
		Mean	1,01±0,33

TABLE 2

Species	No. of invest.	Serotonin concentration, γ/ml
Man	10	0,1±0,07
Dog	29	0,32±0,005
Rabbit	5	2,3±0,19
Guinea pigs	15	0,2
Rats	15	1,3
Mice	25	0,1

blood in these animals, a feature which has often been pointed out by several authors [3, 4], and which was confirmed in our laboratory by M. N. Novikova when studying the morphological composition of the blood of 8 Macacus rhesus monkeys.

The differences found in the content of 5-oxytryptamine in the blood of the Macacus rhesus monkeys (0.70) and the Papio hamadryas monkeys (1.01) were not statistically significant (from the Student-Fisher table, $\alpha = 0.7$).

For comparison, we consider it worthwhile to quote the concentrations of 5-oxytryptamine in the blood of different laboratory animals and in man, as obtained by us, using a variety of methods. These findings are shown in Table 2.

It can be seen from Table 2 that the concentration of 5-oxytryptamine in the blood of monkeys is approximately the same as its level in the blood of rats, which adds to the observations made by other authors [3, 4] that be blood of these animals is morphologically similar.

SUMMARY

Blood serotonin (5-oxytryptamine) content was studied in Macacus rhesus and Papio hamadryas. When comparing the blood serotonin content of various laboratory animals and man it was revealed that the blood serotonin content in monkeys is very close to that in rats.

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THE BIOLOGICAL IMPORTANCE OF AMYLASE TO MUSCULAR ACTIVITY

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In previous research [1, 2] we have shown that the amylolytic activity of the blood during muscular activity in man is increased $1\frac{1}{2}$ to 3 times, and during athletic contests, by 3 to 6 times or more. In connection with these findings, it was of interest to ascertain the character of the changes in amylase activity of the working muscles themselves.

Taking into consideration the reports in the literature [3, 4] on the amylase activity of the muscles in different conditions, we conducted experiments both on whole muscle and on pounded muscle and muscle extracts, in which the amylase and phosphorylase activity of the muscles were determined simultaneously.

EXPERIMENTAL METHOD

Experiments were carried out on the gastrocnemius muscle of the frog. Contraction of the muscle was caused by the electric current from an induction coil, and lasted 7-8 minutes (voltage in the primary coil 1.7 v, from an accumulator). In the circuit was a metronome with a frequency of 60 per minute. The symmetrically opposite muscle acted as a control.

Since the amylase activity of the muscles at 0° was insignificant without preliminary autolysis, we conducted the investigations at room temperature after autolysis for 25-30 minutes. The muscle preparations were incubated with substrate at a temperature of 38-40°. The experiments with whole muscles were conducted without buffered mixtures, since the pH of the objects examined was optimal for amylase activity.

The incubation medium for the aqueous extracts of pounded muscle were prepared as follows: for amylase: 2 ml of extract, 2 ml of 1 % starch solution, 10 mg NaCl, 6 ml water, pH = 6.9-7.0; for phosphorylase: 2 ml of extract, 1 ml of a 0.1 M solution of NaF, 0.5 ml of an M/3 phosphate buffer, 1 ml adenylic acid, 5 ml of a 1% starch solution, 30 mg $MgSO_4$, pH=7.5. In some experiments 1 ml of 0.05 M phloridzin was added to the incubation mixture to suppress the phosphorylase activity.

As index of amylase activity we used the amount of reducing substances, determined by the Hagedorn-Jensen method [6], and of phosphorylase activity — the fall in the amount of inorganic phosphate determined by the Fiske-Subbarow method [5].

EXPERIMENTAL RESULTS

In the experiments with whole muscles it was found that the decomposition of starch per g of working muscle after incubation for 60 minutes was 1.68 times greater, and after 120 minutes, 2.07 times greater than per g of resting muscle.

Similar results were obtained when the muscle was incubated for 120 minutes in physiological saline (Ringer's solution) and the amylolytic activity of the solutions subsequently determined. The physiological saline in which the working muscle was incubated was 1.65 times more active in decomposing starch than the saline in which the resting muscle was incubated. It was therefore concluded that there was a more intensive transfer of amylase into the medium from the working than from the resting muscle.

This confirmed the findings relating to the increase in amylolytic activity of the frog's blood after work by the hindlimb by 1.42 times, and also to the increase in the amylase activity of the aqueous extract of the pounded working muscles of an amputated limb by 1.76 times, whereas in the corresponding, unamputated limb the increase was only 1.43 times by comparison with the amylase activity of aqueous extracts of pounded resting muscles. The addition of phloridzin had no effect on the results of the experiment.

We investigated the phosphorylase activity of aqueous extracts of amputated or unamputated muscles. The results obtained showed that the phosphorylase activity was increased by muscular activity in an aqueous extract of pounded muscles of an unamputed limb by 1.16 times, and of an amputated limb by 1.38 times, by comparison with controls, and moreover, the addition of phloridzin completely inhibited the phosphorylase activity.

Under the influence of work, the phosphorolytic activity was thus increased also, but to a far less extent than the amylolytic activity.

The findings described thus demostrate a considerable increase in the amylase activity of working muscle, and also a more intensive transfer of amylase from the working muscle into the surrounding medium, presumably connected with the increased permeability of the working muscle by comparison with that at rest.

SUMMARY

The amylolytic activity in the frog's gastrocnemius is much higher after exertion than in a muscle performing no work. With the gastrocnemius at work an intensive transfer of amylase and phosphorylase into the surrounding environment is observed, this depending upon the increased muscle permeability during exertion.

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THE ISOLATION OF POLYNUCLEOTIDE PHOSPHORYLASE FROM ANIMAL TISSUES

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In 1955, Grunberg-Manago and Ochoa [6] were able to isolate from extracts of Azotobacter vinelandii a new enzyme — polynucleotide phosphorylase effecting the synthesis of RNA and similar compounds. In 1956, Kornberg et al. [11] showed that an enzyme was present in soluble extracts of Escherichia coli with the property of catalyzing the reaction of polymerization of desoxyribonucleotides. It has further been shown [1,4,8] that polynycleotide phosphorylase is widely distributed in various bacterial cells, yeasts, spinach leaves and so on.

Numerous attempts have made to obtain polynucleotide phosphorylase from animal tissues. Hilmoe and Heppel [10], for instance, were able to show the presence of adeninepolynucleotide phosphorolysis in the presence of an enzyme isolated from the nuclei of the liver cells of a guinea pig. No synthesis of a polynucleotide was obtained, which, in the authors' opinion, depended on contamination of the extract with nucleases. According to the investigations of other authors [3, 5, 9, 13, 14], incorporation of labeled nucleosidephosphates in polynucleotides takes place in extracts of liver, bone marrow, thymus and ascitic cells on the addition of DNA or RNA. Nevertheless there is no direct proof in the literature of the presence in animal tissues of an enzyme which synthesizes polynucleotides.

The aim of the present investigation was to try to find and isolate from animal tissues an enzyme synthesizing polynucleotides.

EXPERIMENTAL METHOD

As test object we selected regenerating liver, thinking that as a result of the accelerated synthesis of nucleic acids in the process of regeneration, increased polynucleotide phosphorylase activity might be found. Partial hepatectomy (removal of the anterior and left lobes of the liver) was performed in white rats weighing 150-200 g. The rats were sacrificed by decapitation 72 hours after operation, the liver was very speedly extracted, washed with cold 0.14 M NaCl and dried on filter paper, cut into several pieces and placed in acetone with dry ice for 10-15 minutes.

Extraction with 0.15 M KCl was then performed (according to data in the literature, potassium ions activate bacterial enzymes [2]) in the course of homogenization for 3 minutes. The homogenate was centrifuged for 1 hour at a rate of 8000 rpm at 4°. The supernatant fluid was dialyzed in the cold against a phosphate buffer (0.01 M, pH=7.4) for 18 hours with constant mixing. The dialyzate was fractionated with ammonium sulfate in the cold. The precipitates were separated by centrifugation under the same conditions and dissolved in buffered phosphate solution containing cystein (0.01 M, pH=6.8). The solution was dialyzed against the same buffer for 4 hours with stirring, or for 20-24 hours when allowed to stand in the cold. We conventionally called this dialyzate enzyme fraction 1.

TABLE 1

Changes in the Content of Adeninenucleotides and Inorganic Phosphate (P_i) in an Incubation Mixture Containing Enzyme Fractions from the Regenerating Liver of Rats

Enzyme fractions	Temperature of incubation, °C	Incubation time, minutes	Amount of compound, in γ/ml of incubation mixt.		Protein content (in mg) in 1 ml of enzyme	Activity of enzyme, units *
			adeninenucleotides	P_i		
I	0	30	2444,0	200,00	26,65	24,7
I	37	30	1785,2	542,24	26,65	24,7
			—658,8	+342,24		
II	0	30	2483,6	42,48	3,67	57,0
II	37	30	2274,4	129,00	3,67	57,0
			—209,2	+86,52		

* The activity of the enzyme was determined by the fall in the adeninenucleotides in γ/30 min·mg of protein.

TABLE 2

Changes in the Content of Adeninenucleotides, P_i and Readily Hydrolyzed Phosphorus in the Presence of Enzyme Fraction II

No. of enzyme preparation	pH of incubation mixture	Incubation time minutes	In γ/ml of incubation mixture *			Protein content (in mg) in 1 ml of enzyme	Activity of enzyme, units **
			decrease in adenonucleotides	increase in P_i	decrease in readily hydrolyzed P		
1	8,1	15	330,0	50,6	46,6	3,67	90,0
1	8,1	30	209,0	85,0	83,0	3,67	90,0
2	8,2	15	1466,0	34,6		7,82	187,0
2	8,2	60	109,0	85,5		7,82	187,0
3	7,2	15	606,0	24,9	30,3	3,71	163,0

* The numbers given represent the difference between the results of the experimental (incubation at 37°) and control tests (incubation at 0°).

** The activity of the enzyme was determined by the fall in the adeninenucleotides in γ/15 min·mg of protein.

EXPERIMENTAL RESULTS

In preliminary experiments we judged the activity of the enzyme by the increase in inorganic phosphate in the incubation mixture, bearing in mind that synthesis of the polymer from adenosinediphosphates proceeds according to the following scheme [5]:

$$n\text{-}A-P-P \rightleftharpoons (A-P)_n + n\text{-}Pi$$

where A — adenosine and Pi — inorganic phosphate.

Subsequently, however, because of the presence of phosphatase, especially in enzyme fraction I, in addition to determining the inorganic and readily hydrolyzed phosphorus, we measured the quantity of

adeninenucleotides in the acid-soluble fraction obtained by addition of chloric acid to the incubation mixture. As substrate for the reaction we used a preparation of ATP containing 20% of ADP as contaminant. The reaction mixture also contained $MgCl_2$, EDTA (ethylenediaminetetra-acetic acid), glycyl-glycine-tris buffer (pH = 7.2 and 8.2) and the enzyme.

The adeninenucleotides were estimated spectrophotometrically at 290 mμ and 260 mμ, and the inorganic and readily hydrolyzed phosphorus by a colorimetric method (Fiske-Subbarow). In some cases the decrease in the adenine concentration in the acid-soluble fraction after hydrolysis in chloric acid was estimated by the methods of paper chromatography and spectrophotometry.

Further purification of the enzyme was carried out with zinc chloride and ethyl alcohol. To 58 ml of dialyzate, acidified with acetic acid, was added 0.35 ml of 0.5 M $ZnCl_2$. The precipitate was removed by centrifugation in the cold, and to the supernatant fluid was added cold 50% ethyl alcohol, in a volume of 7.7 ml alcohol to 35 ml of centrifugate. After centrifugation for 5 minutes at 8000 rpm in the cold, the residue was dissolved in tris buffer (0.05 M) (enzyme fraction II).

This purification led to an increase in the activity of the enzyme, as determined by the fall in the concentration of adeninenucleotides in the process of incubation, by roughly 2.3 times (Table 1).

By a method described later, we isolated three enzyme preparations, and they all possessed this degree of activity (Table 2).

It will be seen from Table 2 that incubation of the reaction mixture in the presence of enzyme fraction II led to a considerable decrease in the content of adeninenucleotides and readily hydrolyzed phosphorus and to an increase in the inorganic phosphate.

It must be mentioned that during incubation, a decrease in the adeninenucleotide content of the acid-soluble fraction was observed in the first 15 minutes, and as the time of incubation was prolonged, these changes were no longer observed.

This is to some extent in agreement with the findings of Littauer and Kornberg [12], who showed that in the original and insufficiently purified preparations of polynucleotide phosphorylase from E. coli, accumulation of the polymer took place in insignificant amounts, and as the time of incubation was prolonged, synthesis of polynucleotides ceased.

We showed by a special series of control experiments that no changes took place in the test objects in the absence of substrate (ATP and ADP) and also in the absense of enzyme. Heating the enzyme fractions at 100° for 1 minute completely inactivated them. The enzyme preparations were unstable on keeping. On the 7th day of storage at temperatures of 0° and −10°, the activity of the enzyme fractions disappeared.

These afforded only indirect evidence of the possible presence in the different fractions of the regenerating rat liver homogenates of polynucleotide-synthesizing enzymes. In this connection investigations were made in order to detect an increase in the adeninenucleotide content of the acid-insoluble fraction, and thereby to exclude the possible action of adeninenucleotide deaminase. Preliminary experiments showed that enzyme fraction I of regenerating rat liver possesses the power of increasing the content of acid-insoluble product during incubation (by increasing the adeninenucleotides in the acid-insoluble fraction). These findings will be discussed at greater length in subsequent communications.

SUMMARY

The presence of enzyme fractions isolated from the regenerating rat liver gives rise to the following changes during incubation with adenosinephosphates: increase in organic phosphorus content, reduction in easily hydrolyzed phosphorus and adeninenucleoprotein in the acid-soluble fraction and augmentation of the adeninenucleoprotein concentration in the acid-insoluble fraction.

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PHARMACOLOGY

THE EFFECT OF ANALGESICS ON THE CORONARY CIRCULATION

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Analgesics are widely used in clinical practice in treatment of coronary insufficiency, being required in the eradication of pain accompanying attacks of angina pectoris. The changes caused by analgesics in the blood suppy of the myocardium are, therefore, extremely important.

The literature on the effect of analgesics on the coronary circulation is confined to a few experimental investigations dealing with morphine. Even these few findings were obtained, however, mainly in experiments on the isolated heart, and they are distinctly controversial. Kountz [9], for instance, in experiments on the isolated surviving human heart, observed an increased outflow from the coronary vessels under the influence of morphine. A similar conclusion was reached by Elek and Katz [8] on the basis of experiments on the isolated fibrillating heart. Van Egmond [13] and Wegria [14] showed that morphine causes no essential changes in the coronary blood flow.

According to the findings of a number of authors, phenadon and promedol possess spasmolytic properties. The spasmolytic activity of these drugs in mainly shown, however, in experiments on isolated organs (diminution of the spastic contractions od isolated segments of the gut and dilation of the vessels of the isolated ear of the rabbit, and so on). In the intact animal, promedol and phenadon lead, on the other hand, to an increase in the tone of the smooth muscle of the uterus and gut [3-6]. The question of the spasmolytic properties of these analgesics is thus by no means clear, and in particular their action on the coronary circulation has not been investigated.

The object of the present research was to study the action of analgesics on the coronary circulation. For the investigation we chose the following drugs: thecodin, promedol and phenadon.*

EXPERIMENTAL METHOD

Experiments were conducted on cats anesthetized with urethane and chloralose. The blood supply to the heart was judged by the volume of blood flowing from the coronary sinus in unit time. A detailed description of the method has been given in our previous communication [1]. In order to assess more objectively the influence of the drugs under study on the vessels of the heart, in a series of experiments we recorded the tone of the coronary vessels by means of a perfusion pump, the perfusion volume being constant. The construction and working principle of this apparatus were described by V. M. Khayutin, V. M. Danchakov and V. L. Tsaturov [7]. The advantages of this method are that it permits the state of the vascular tone to be judged independently of the influence of the level of the systemic blood pressure on the vessels of the area being studied. In order to record the tone of the coronary vessels by the autoperfusion method, a special cannula was passed from the subclavian artery, through the arch of the aorta into the orifice of the left common coronary artery, under artificial respiration after thoracotomy. The perfusion pump collects blood from the carotid artery and injects it at a constant perfusion volume into the coronary vessels. The perfusion pressure, recorded by a mercury

* Amidone.

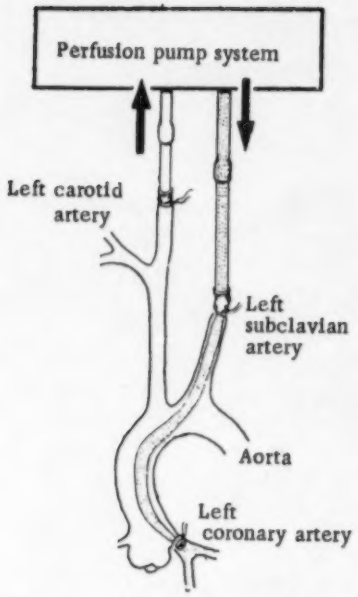

Fig. 1. Scheme of autoperfusion of the left coronary artery of the heart in the cat.

manometer at the outlet of the pump, is determined by the resistance of the vessels of the heart and is an index of their tone. The scheme of the experimental autoperfusion of the coronary vessels by means of the perfusion pump is illustrated in Fig. 1.

The blood pressure was recorded in the femoral or carotid arteries. To prevent the blood from clotting, heparin was injected (1000-1500 units/kg). The test drugs were injected intravenously.

EXPERIMENTAL RESULTS

The experiments showed that morphine possesses the property of increasing the volume flow of the coronary circulation. In doses of only 1-2 mg/kg it led to an increase in the outflow of blood from the coronary sinus by 20-30% by comparison with its original level. Concurrently with an increase in the rate of blood flow, a fall of 25-30 mm Hg in the blood pressure was observed. When larger doses of morphine were given (3-4 mg/kg), the outflow of blood from the coronary sinus increased by 50-60% over the initial level (Fig. 2, a). The effect lasted from 10 to 15 minutes, depending on the dose of the drug.

The experiments in which the coronary vessels were perfused showed that after injection of morphine a marked fall in the perfusion pressure was observed, giving evidence of a decrease in the resistance of the vessels of the heart to the blood flow (see Fig. 2, b). The increase in the blood supply of the heart under the influence of morphine was evidently connected with its direct effect on the tone of the heart vessels. The experiments with morphine thus showed that the drug had a positive action on the coronary circulation, lowering the tone of the coronary vessels.

We obtained quite different results in the experiments with promedol. In the majority of experiments, promedol in doses of 1-2 mg/kg caused a decrease in the volume rate of the coronary blood flow by 15-20% by comparison with the original level. In only a few experiments when promedol was given in these doses were no essential changes observed in the magnitude of the outflow of blood from the coronary sinus. As the result of an increase in the dose of promedol to 3 mg/kg, the decrease in the rate of the coronary blood flow reached 40-45% (Fig. 3, a). In response to injection of promedol, as a rule a fall in the blood presure of 15-25 mm Hg was observed. It might have been assumed that the fall in the rate of blood flow was not, in this case, an index of constriction of the coronary vessels, but merely pointed to the fact that when the level of the blood pressure was lowered, a smaller volume of blood passed through the vessels of the heart in unit time. However, experiments in which the tone of the vessels of the heart was recorded showed that under the influence of promedol the resistance of the coronary vessels was, as a rule, increased (see Fig. 3, b).

We obtained analogous results in experiments with phenadon, which, in doses of 1-2 mg/kg, led in the majority of cases to a decrease in the volume rate of the coronary blood flow by 25-60% by comparison with the initial level. The duration of its action in these conditions was 15-25 minutes. In some experiments, however, after injection of phenadon no changes were observed in the magnitude of the outflow of blood from the coronary sinus. The results of the experiments on the study of the tone of the vessels of the heart under the influence of phenadon were in agreement with the findings obtained when the outflow of blood from the coronary sinus was recorded. When the coronary vessels were perfused during the action of phenadon, in roughly half the experiments the tone of the vessels was increased, whereas in the remaining cases no essential changes in the tone of these vessels could be found.

Thecodin in doses of 1-2 mg/kg caused no perceptible changes in the volume rate of the coronary blood flow. In some experiments a fall in the outflow of blood from the coronary sinus, insignificant (by 15-20%) by comparison with the initial level, was observed and this, as shown by special experiments, was evidently not connected with the influence of thecodin on the tone of the coronary vessels.

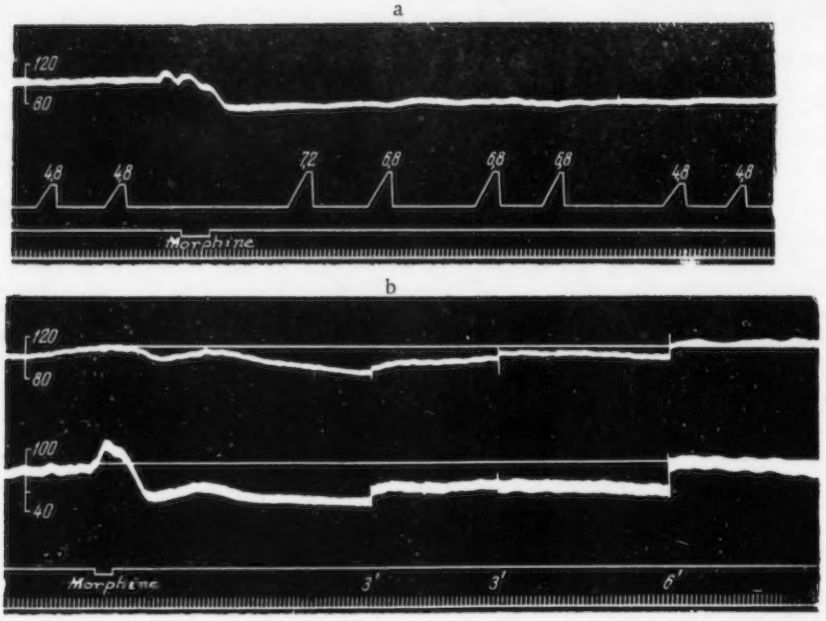

Fig. 2. The effect of morphine on the volume rate of outflow of blood from the coronary sinus (a) and on the tone of the coronary vessels (b). Significance of the curves (from above down): a) blood pressure, outflow of blood from the coronary sinus in ml/minute (height of the column — blood flow in 15 seconds, figures above the columns — minute volume of blood flow), marker of injection of morphine (3 mg/kg), time marker (5 seconds); b) perfusion pressure, reflecting the resistance of the vessels of the heart and its initial level, blood pressure and its initial level, marker of injection of morphine (2 mg/kg), time marker (5 seconds).

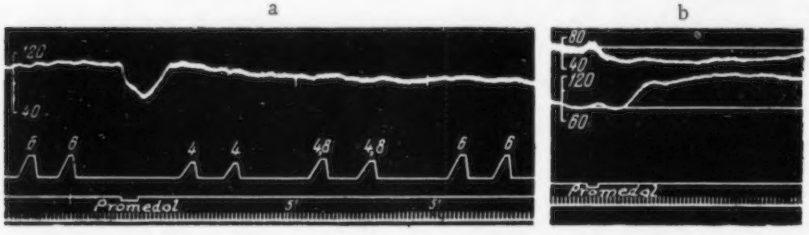

Fig. 3. The effect of promedol on the volume rate of outflow of blood from the coronary sinus (a) and on the tone of the coronary vessels (b). Significance of the curves as in Fig. 2, a and b respectively. The dose of promedol injected in (a) was 3 mg/kg, and in (b) 2 mg/kg.

The experiments thus showed that, of the analgesic drugs tested, only morphine possessed the power of augmenting the blood supply to the heart. Promedol and phenadon led in the majority of cases to a decrease in the volume rate of the coronary blood flow and to an increase in the tone of the coronary vessels. Thecodin caused no essential changes in the state of the coronary circulation.

Our findings do not, it seems, support certain clinical observations, according to which analgesics (for example, promedol) are effective in the treatment of angina pectoris [2 and others].

According to M. Yu. Ladinskaya's observations, however, analgesics lead to normalization of the

electrocardiographic changes caused by closure of the lumen of one of the coronary arteries. A temporary restriction of the blood flow in one of the coronary arteries may, we know, cause the appearance of reflex expressed by the development of spasm of the other vessels of the heart [10-12]. Taking these findings into consideration, it may be considered that the effectiveness of the majority of analgesics in angina pectoris is due not only to the suppression of the pain syndrome but also to suppression of these reflex reactions just described, and has nothing to do with the direct action of these drugs on the vessels of the heart.

Our findings, which indicate the possible aggravation of the blood supply of the myocardium by the use of promedol and phenadon, must be taken into consideration in clinical practice.

SUMMARY

The author studied the effect of analgesics (morphine, thecodin, phenadon and promedol) on the blood output from the coronary sinus and the tone of the coronary blood vessels in cats. Of the analgesics mentioned only morphine increases the cardiac blood supply. In the majority of cases the introduction of promedol and phenadon leads to a decrease of the volume velocity of the coronary blood flow, increasing the tone of coronary vessels. Thecodin exerts no material effect on the coronary circulation.

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THE ACTION OF PHOSPHOTRIOSE, PHOSPHO-ENOLPYRUVIC ACID AND THE PREPARATION ZSC (ZYMOSTIMULATOR CORDIS) ON CARDIAC MUSCLE

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In previous published work carried out jointly by T. M. Tupaev and ourselves [5], it was shown that preparations of "sodium-DPF" — the sodium salt of fructose 1,6-diphosphate in concentrations of 1: 2000 and over, in contrast to the free sugars (glucose and fructose), and also preparations of synthetic glucose 6-phosphate and glucose 1-phosphate, have a prolonged and marked stimulating action on the contraction of the heart muscle of the frog, when weakened by continued work in Ringer's solution.

Subsequently, by means of absorption on carbon, fractionation of the barium salts isolated from the eluate and chromatography of one of the fractions, we were able to isolate from the sodium-DPF preparations, and also from a yeast fermentation mixture, a substance which had a stimulating action on the work of the heart, in a concentration of 10^{-5}, and which we called ZSC (zymostimulator cordis) until its structure has been fully established. A study of the chemical nature of this compound has shown that it closely resembles or is identical with uridinediphosphate (UDP [4]).

Preparations of sodium-DPF, free from ZSC, had no appreciable action on the strength concentration of the isolated frog's heart in the ordinary conditions previously described [5], although clinically they had an antishock action [4].

It appeared of interest to continue the investigation of various phorphorylated products of carbohydrate-phosphorus metabolism in respect to their action on the heart. For this purpose, in the present research we prepared and tested the action on the heart of triose phosphate compounds — products of the aldolase decomposition of DPF and high-energy compounds — phospho-enolpyruvic acid.

EXPERIMENTAL METHOD

Triose phosphate compounds were prepared mainly by the method of Meyerhof and Lohmann [6], by decomposition of DPF (sodium salt) with aldolase. The source of aldolase which was used was a dialyzed extract of rabbit muscle. The triose phosphates were isolated and purified in the form of their barium salts. As in the original work [6], the triose-phosphate phosphorus accounted for about 90% of the total organically combined phosphorus of the preparation: 56.4% of the triose phosphate was in the form of 3-phosphoglyceraldehyde and the remainder phosphodioxyacetone.

Bearing in mind the possibility of contamination of the compounds obtained with highly active substances (for example, of the ZSC or ATP type), we studied the action of the biochemically prepared compounds both before and after treatment with carbon at room temperature to absorb contaminants. The resulting preparation of the barium salt was divided into 3 parts, one of which was not treated with carbon, the second was treated with carbon for one hour at pH 3, and the third, after precipitation of the Ba with sodium

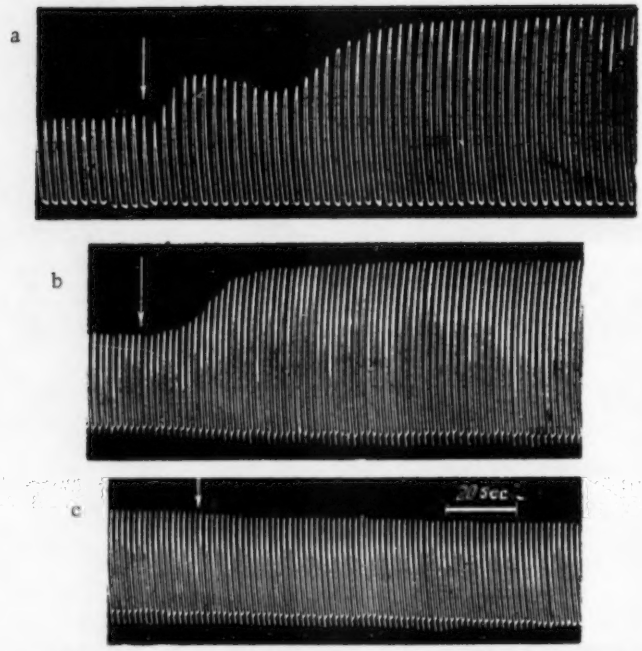

Fig. 1. The action of the triose phosphate preparations in a concentration of 0.05% on the frog's heart. a) Before treatment with carbon; b) after treatment with carbon at pH 9; c) after treatment with carbon at pH 3. The arrow indicates the moment of injection of the solution of the preparations into the cannula.

sulfate, was treated with carbon at pH 9 (also for one hour). For the biological trials we prepared basic 1% solutions of the sodium salt of the triose phosphates (untreated with carbon, and treated at pH 3 and pH 9), from which less concentrated solutions were prepared by the addition of suitable amounts of diluent.

Phospho-enolpyruvic acid (PEP) was prepared by biochemical and chemical methods.

Biochemically, PEP was prepared by the method of V. A. Belitser and T. V. Saenko [2] from the sodium salt of 3-glycerophosphoric acid, which is converted by the action of the yeast enzymes phosphoglyceromutase and enolase into PEP. The raw material — 3-glycerophosphoric acid (GPA)-was prepared by Ostern and Guthke's modification of Neuberg and Kobel's method, taking into consideration the findings of Belitser and Karlina [1] and Severin and Meshkova [3], by oxidation of triose phosphates (formed from DPF) with acetaldehyde in the presence of sodium fluoride. GPA was isolated in the form of the acid barium salt with two molecules of water of crystallization. The product contained 91-93 % of the crystalline hydrate of the barium salt of GPA (ignoring hygroscopic moisture).

PEP, obtained biochemically from GPA, was isolated in the form of the barium-silver salt, which was then converted into the barium and sodium salt. The biochemically prepared PEP, as in the experiments with triose phosphates, was used either untreated with carbon or treated with carbon at pH 3 and pH9.

PEP was synthesized chemically by Ohlmeyer's method, by phosphorylation of pyruvic acid in anhydrous quinoline by means of phosphorus oxychloride. Synthetic PEP was isolated in the form of the crystalline barium-silver salt. After two or three recrystallizations a preparation was obtained containing about 92% of the barium-silver salt of PEP (ignoring hygroscopic moisture). All the phosphorus in the preparation was organic and belonged to the PEP, since it was completely split off with iodine.

For the biological trials, the barium-silver salt of the synthetic PEP was converted, by the addition of Na_2SO_4 and HCl (with subsequent neutralization), into the sodium salt, which was prepared in the form of an

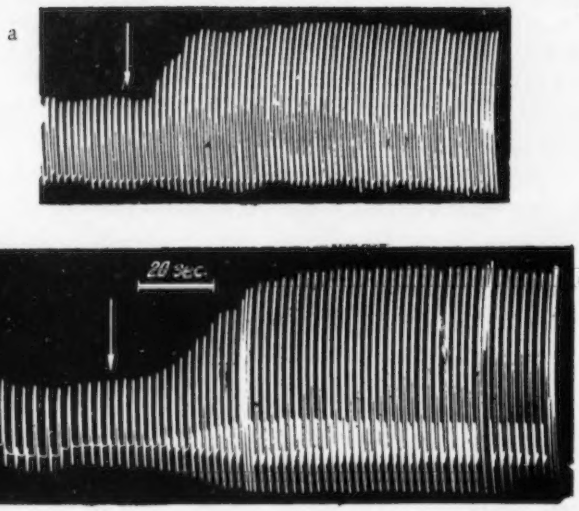

Fig. 2. The action on the frog's heart of eluates from carbon after treatment of triose phosphates with carbon at pH 3 (a), and of a solution of ZSC in concentration of 10^{-6} (b).

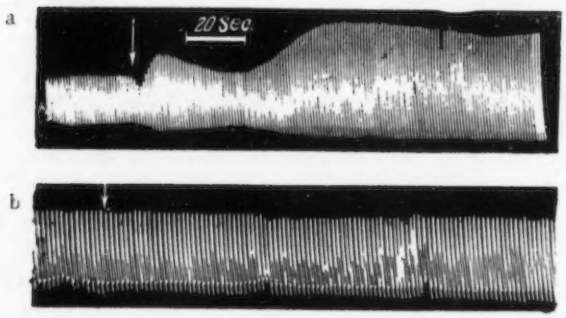

Fig. 3. The action of biochemically prepared PEP, in a concentration of 0.1%, on the frog's heart. a) Before treatment with carbon; b) after treatment with carbon at pH 3.

original 1% solution, as in the experiments with the biochemically prepared PEP. Before the experiment, all the test substances were diluted with Ringer's solution to the required concentrations.

EXPERIMENTAL RESULTS

Neutral solutions of the sodium salts of the triose phosphates were tested on the frog's heart, isolated by Straub's method, the contractions of which were greatly depressed in amplitude by prolonged working (1-2 days) in a humid chamber at a temperature of 2-4°.

The triose phosphates which were not treated with carbon, in concentrations of 0.001 and 0.01%, had no or a comparatively weak action on the contraction of the heart muscle. In concentrations of 0.05-0.1%, however, a very obvious effect was observed (Fig. 1, a): the amplitude of the cardiac contractions was sharply increased and this action lasted about 30 minutes.

Both by the character of its action (the presence of three phases—an initial rise, then a fall in the amplitude of the contractions and a subsequent prolonged increase in the amplitude of the cardiac contractions), and by the magnitude of the effective doses, the triose phosphate preparations untreated with carbon closely resembled the previously studied DPF preparations, also untreated with carbon [5], although they were slightly inferior to then in respect to the action of small doses.

The triose phosphate preparations treated with carbon at pH 9 possessed practically the same intensity of activity as the untreated preparations, but the character of their action was altered: the three-phase character had disappeared and was replaced by an even increase in the amplitude of the contractions (Fig. 1, b).

The triose phosphate preparations treated with carbon at pH 3 were completely inactive (Fig. 1,c).

In order to discover to what extend the triose phosphates were preserved when treated as described above in an alkaline or acid medium, analyses were made of the triose phosphate content (as alkaline-labile and total phosphorus). As was to be expected, the triose phosphate content of the preparations treated with carbon at pH 9 had fallen by approximately 35-40% of the initial value. In the preparations treated at pH 3, and having completely lost their activity, no fall in the triose phosphate content was observed.

These facts led to the conclusion that the action on the heart (under these conditions of testing) was a property not of the triose phosphates themselves, but of the contaminants absorbed on the carbon.

Since the triose phosphate preparations were obtained from DPF, it was natural to asume that they might contain primarily the same contaminants as were present in the DPF, including ZSC, especially as in the subsequent work there was no treatment which would completely remove ZSC.

For confirmation of the hypothesis that ZSC was present in the triose phosphate preparations after adsorption of contaminants on carbon at pH 3, the carbon was treated with water alkalized with NaOH to pH 9, as was done in the isolation of ZSC. The eluate obtained was tested on the heart after neutralization, and showed high activity with a monophasic rise in the amplitude of the cardiac contractions (Fig. 2 a).

Finally, by means of the method described above, triose phosphates were prepared from DPF which had been preliminarily treated by our method [4] to free it from ZSC. These triose phosphates were found to be inactive.

A study of the ultraviolet light absorption of the eluate from the carbon used in treatment of the triose phosphates showed the presence of uridine derivatives, since the peak at 260 mμ fell sharply after treatment with bromine.

The action of the triose phosphate preparations on the isolated heart was thus due primarily to the presence of ZSC as a contaminant, but this does not, of course, exclude the posibility of other active contaminants, on the investigation of which we are at present engaged.

Fig. 2 shows the kymogram of the action on the heart of chromatographically homogeneous ZSC, prepared by the method described previously [4], in a concentration of 10^{-5}. A noteworthy feature is the monophasic character of the action — an even increase in the amplitude of the contractions-which distinguishes it sharply from ATP, which is characterized by a three-phase action.

The change in the character of the action of the triose phosphate preparations treated with carbon at pH 9 (disappearance of three-phase character) may evidently be accounted for by adsorption of a substance with a three-phase action (perhaps ATP on the carbon, or by destruction of some contaminant at this pH.

This problem is being studied at the present time. The three-phase character of the action of the triose phosphate preparations is evidently the sum of the actions of ZSC, with a monophasic action, and of a substance with a three-phase action (probably ATP).

The monophasic character of action of the eluate obtained by treatment with carbon at pH 9 (after treatment of triose phosphates with this carbon at pH 3) is evidently explained by removal from the carbon of ZSC alone, and retention on the carbon of the substance with a three-phase action, for example ATP, which possesses more basic properties than the ZSC.

Neutral solutions of preparations of the sodium salt of PEP, obtained by a biochemical method, showed a constant and persistent three-phase effect on the contractions of the isolated heart, like that of the triose phosphates, in concentrations of 0.05% and over (Fig. 3, a). Biochemically prepared PEP, treated with carbon at pH 9, possessed the same degree of activity; the preparation treated with carbon at pH 3 was inactive (Fig. 3,b).

Analysis of the two preparations showed that treatment at pH 3 and pH 9 causes only a slight fall or practically none at all (within limits of 10%) in the content of PEP. The eluate from the carbon used in the treatment of the PEP at pH 3 possessed high activity on the heart.

PEP prepared by chemical synthesis, in a concentration of 0.05%, was found to be inactive; higher concentrations (above 0.1%) of the synthetic PEP, just as of that prepared biochemically, depressed the activity of the heart*.

* We express our deep gratitude to T. M. Turpaev for advice in the physiological testing of our preparations.

All these phenomena may evidently be explained just as in the case of the triose phosphates: the activity of the biochemically prepared PEP is primarily due to the action of ZSC as a contaminant, present in the yeast.

It seems to us that a fact of great interest is that PEP itself (synthetic, or prepared biochemically and purified), being a high-energy compound, had no stimulating action on the contraction of the cardiac muscle under these conditions of testing.

In this connection it must be remembered that recently Moos and Lorand [7], in a study of the action of synthetic PEP on a far simpler system — fibers of the psoas muscle treated with glycerol — showed that PEP causes relaxation of the fibers after the action of ATP.

SUMMARY

Preparations of the sodium salt of triose phosphate , obtained by biochemical means, as well as those of the sodium salt of phospho-enolpyruvic acid (PEP) obtained both biochemically and by chemical synthesis were studied in respect to their action upon the fatigued frog's heart (by Straub's method). Synthetic PEP was found to be inactive, while triose phosphate and biochemically obtained PEP exercise a stimulating effect on the heart similar by its character and its effective dose to the action of the sodium salt of fructose-1,6-diphosphate (DPF).

Treatment of triose phosphate preparations (obtained biochemically) and those of PEP with charcoal at various pH, and their subsequent examination along with that of the eluted fluid (wash off) were investigated. It was demonstrated that the action of the preparations on the fatigued heart is, at least in its main part, associated with the presence of the low quantities of zymostimulator cordis (ZSC) as admixture. ZSC is a uridine derivative close or identical to uridinediphosphate (UDP) formerly isolated by the authors from yeast and sodium-DPF preparations.

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THE MECHANISM OF BRADYCARDIA CAUSED BY CARDIAC GLUCOSIDES

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(Received July 7, 1958. Presented by Active Member AMN SSSR V. V. Zakusov)

Bradycardia is one of the characteristic signs of the action of the cardiac glucosides. The mechanism of its production, despite the abundant work on the subject, remains, however, unexplained and debatable. This is revealed by the contradictory results described in the literature. Several authors explain the bradycardia by the influence of the cardiac glucosides on the vagus nerve centers [8, 9, 18]. Others point out the sensitization of the myocardium to the influence of the vagus and to the action of acetylcholine [4, 16] or the sensitization of the intramural ganglia to acetylcholine [15]. The majority of research workers regard bradycardia as a cardiocardial reflex [5, 6, 13 and others], although these authors present no direct proof of the reflex mechanism of this action*.

With the possibility of using a new method of transplantation of the heart, suggested by V. P. Demikhov, we carried out the present research for the purpose of studying the mechanism of bradycardia caused by the cardiac glucosides.

EXPERIMENTAL METHOD AND RESULTS

Experiments were conducted on 12 dogs in which the heart was transplanted by V. P. Demikhov's method. As a result of the transplantation, the animal, after operation, had two hearts: one — its own (with its innervation intact), the other — the grafted heart, "denervated" in the sense of exclusion of central influences. In dogs such as these we studied the action of strophanthin-g and of a standard extract of digitalis in therapeutic and toxic doses. The activity of the heart was recorded by means of a Hürtle's manometer from the brachial arteries of the animal, and in some experiments electrocardiographically. The experiments were performed on dogs under morphine-sodium amytal anesthesia on the day of operation, and on controls on the second day. The results of the investigation enabled the direct influence of the cardiac glucosides on the intramural ganglia of the heart, the M-cholinoceptors** and the myocardium to be elucidated.

It was shown by the experiments on dogs with two hearts that the dog's own heart and the denervated heart reacted differently to injection of the cardiac glucosides. Strophanthin-g, for instance, when injected intravenously in a dose of 50 μg/ kg, caused bradycardia of the dog's own heart. In the course of time the bradycardia increased, and the rate then gradually returned to the original value. Under these circumstances the rate of the denervated heart was quite unchanged.

* A detailed survey of the literature was given in the article by V. V. Zakusov in the journal "Farmakologiya i Toksikologiya", No. 1 (1957).

** Receptors of the parasympathetic system.

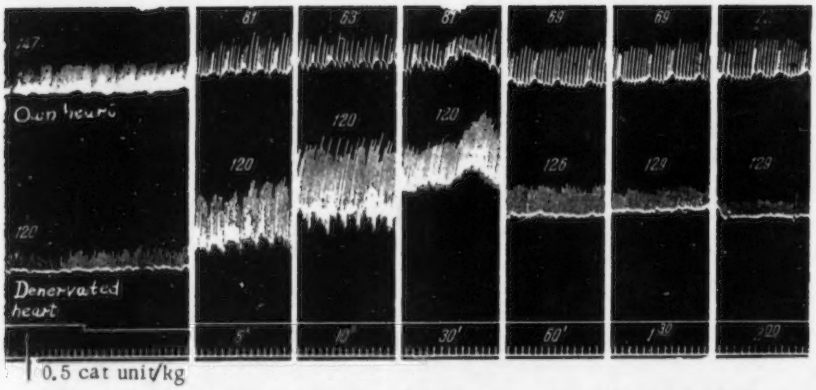

Fig. 1. The effect of a standard extract of digitalis in a dose of 0.5 cat unit/kg on the rhythm of cardiac contractions. Significance of the curves (from above down): record of the contractions of the dog's own heart· of the denervated heart; injection marker; time marker (2 seconds).

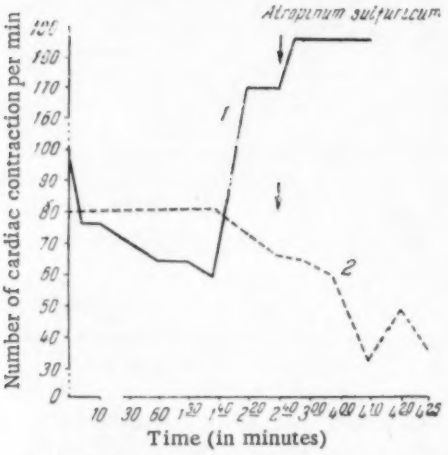

Fig. 2. Change in the rhythm of the heart in response to the action of strophanthin-g (0.15 mg/kg). 1) Dog's heart; 2) denervated heart.

A similar picture was observed in response to injection of a standard extract of digitalis in a dose of 0.3-0.5 cat units/kg (Fig. 1).

It may be seen from the kymogram (see Fig. 1) that, under the influence of this preparation, a marked bradycardia of the dog's own heart developed sharply, in individual experiments falling to 40% of the original rate, whereas the rhythm of the denervated heart remained unchanged. It must be mentioned that the rate of the denervated heart subsequently became slightly quickened and extrasystoles appeared, evidently on account of the muscular action. The amplitude of both the dog's own heart and the denervated heart rose considerably during the action of the cardiac glucosides.

In toxic and lethal doses, strophanthin-g caused in the dog's own heart the typical picture of the action of the cardiac glucosides (Fig. 2).

For instance, after injection of strophanthin in a dose of 0.15 mg/kg, in the first period of action of the drug a considerable bradycardia of the dog's own heart developed, to be replaced later by an increase in the rate and then by cardiac arrest. The rhythm of the denervated heart was, as a rule, unchanged in response to the injection of lethal doses of the glucoside during the first hour, and a well-marked slowing of the rhythm then appeared. In some experiments the bradycardia was temporary in character and rapidly changed into tachycardia and arrhythmia.

In order to ascertain the character of the bradycardia developing in response to lethal doses of cardiac glucosides, the dog was given an intravenous injection of atropine sulfate in a dose of 0.5 mg/kg, and of hexamethonium in a dose of 5 mg/kg. In these cases atropine caused a quickening of the rate of the dog's own heart and had no effect on that of the denervated heart. Hexamethonium, like atropine, did not abolish the bradycardia of the denervated heart. The standard extract of digitalis in a dose of 1 cat unit/kg caused similar changes in the rhythm.

Histological examinations of the heart of dogs dying from lethal doses of strophanthin-g, carried out by M. F. Bystrova, showed that after injection of this drug, well-marked vascular changes were observed in the

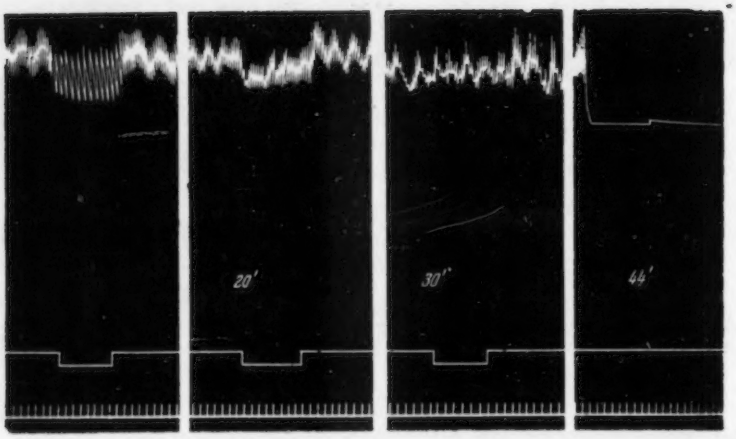

Fig. 3. The influence of strophanthin-g on the transmision of excitation from the vagus nerve to the heart muscle of the cat, during continuous intravenuous infusion of the drug in a concentration of 1: 200,000. Significance of the curves (from above down): recording of the cardiac contractions of the cat; stimulation marker; time marker (1 second). The figures above the stimulation marker correspond to the time from the start of infusion of the strophanthin solution.

myocardium — venous congestion, perivascular edema and hemorrhages, swelling and desquamation of the endothelium, plasmorrhoea and, in individual cases even necrosis of the walls of the arteries, moderate cellular proliferation of the stroma around the vessels, loosening of the endocardium, focal proliferation of the endothelium; scattered subendocardial hemorrhages, in individual experiments the muscle fibers of the myocardium were stained unevenly and granular dystrophy of the protoplasm was observed.

In addition to the investigations on dogs with two hearts, we carried out experiments on cats, in which the transmission of excitation from the vagus nerves to the heart muscle was recorded during continuous infusion of strophanthin in a concentration of 1: 200,000 until arrest of the heart. During the performance of this series of experiments the peripheral end of the vagus nerve was stimulated by means of an electronic stimulator with a frequency of 30 cps, the duration of a single stimulus being 0.5 millisec. The experiments showed that under the influence of strophanthin, the transmission of impulses was impeded; with an increase in the dose the block became more marked, and at doses of 0.5-0.7 cat unit/kg the transmission of impulses ceased altogether (Fig. 3).

To explain the action of the cardiac glucosides on the vagus nerve center, experiments were conducted on dogs with crossed circulation (5 experiments). Both carotid arteries and jugular veins of the donor dog were sutured to the two carotid arteries and jugular veins of the recipient dog. The spinal cord of the recipient dog was as a rule divided or both vertebral arteries were ligated. Blood from the donor dog entered the head of the recipient dog, which was connected to the trunk only by means of the two vagus nerves; the humoral effects of the cardiac glucosides were excluded from the heart of the recipient dog. The activity of the hearts was recorded manometrically, and strophanthin was injected into a vein of the donor dog. Injection of strophanthin in therapeutic and even lethal doses, causing bradycardia and cardiac arrest in the donor dog, had no effect on the rhythm of the heart of the recipient dog, which showed that the cardiac glucosides had no central action.

From an analysis of the experimental findings it may be concluded that the reaction of the dog's own heart and of the denervated heart to injection of cardiac glucosides differed. A standard extract of digitalis and strophanthin-g in therapeutic doses caused a well-marked bradycardia of the dog's own heart, but did not affect the rhythm of the denervated heart. The absence of bradycardia of the transplanted heart gives grounds for the belief that bradycardia during administration of cardiac glucosides is not the result of their stimulating action on the intramural ganglia of the heart and the M-cholinoceptors. The absence of a stimulating action of the cardiac glucosides on the M-cholinoceptors was confirmed by experiments on cats in which the

transmission of excitation from the vagus nerves to the heart muscle was recorded; it was shown in these experiments that strophanthin blocked the transmission of impulses.

Our experimental results are in agreement with the findings of V. V. Zakusov that the transmission of impulses from the vagus nerve to the heart muscle is impeded by the action of the cardiac glucosides. This view is confirmed by the work of a number of investigators [10, 12, 17, 19 and others], who found no enhanced effect from stimulation of the vagus nerves after injection of digitalis and strophanthin.

The experiments thus showed that the cardiac glucosides possess no stimulating action on the intramural ganglia of the heart nor on the M-cholinoceptors. We are left, therefore, with the suggestion that the bradycardia in response to the action of the cardiac glucosides may be due to their influence on the vagus center, or it may be produced reflexly, as a cardiocardial reflex. Experiments on dogs with crossed circulations reveal that the cardiac glucosides have no central stimulating action. Similar conclusions were reached by J. F. Heymans and C. Heymans[11] on the basis of their experiments on dogs. The experiments ofGreen and Peeler [9] on excitation of the vagus center cannot be regarded as affording relevant evidence, for they were carried out only on tortoises, and the authors used large doses of digitalis. The work of D. A. Kharkevich and L. A. Kitaev showed that strophanthin-k stimulates the vagus nerve center in cats only when large, unphysiological doses are used.

It follows from these experiments that the cardiac glucosides have no stimulating action on the ganglia of the heart, the M-cholinoceptors and the vagus nerve center. This gives ground for the belief that, in the development of bradycardia caused by cardiac glucosides, reflex mechanisms of action are of decisive importance. The bradycardia develops as a cardiocardial reflex, like that established by Bezold in relation to veratrin [7]. When lethal doses of cardiac glucosides are given, the bradycardia of the denervated heart is evidently due to the action of the drugs on the myocardium, since it is not abolished by atropine or hexamethonium. This is also confirmed by histological investigations, which reveal considerable myocardial damage at these doses. In this connection the work of Loubatières and Sassine [14] is of interest, showing that in the isolated auricle of the rabbit's atrium, after exclusion of synaptic structures with hexamethonium, strophanthin causes a slowing of the rhythm; this is proof of its direct action on the cardiac muscle of the auricle.

SUMMARY

V. P. Demikhov's new method of heart transplantation, used for the first time in pharmacological analysis, was employed to study the mechanism of the action of cardiac glucosides. Experiments were conducted on dogs with two hearts: one of them — their own, and another — transplanted, "denervated" i.e., with exclusion of influences from centers). Investigations demonstrated that cardiac glucosides have no exciting effect on the heart ganglia and M-cholinoceptors. This is confirmed by experiments on cats, which showed that under the effect of strophanthin-g the impulse transmission from the vagus nerve to the heart muscle is inhibited. Experiments with cross circulation on dogs demonstrated the absence of any exciting effect of the cardiac glucosides on the vagus center. Hence, under the action of cardiac glucosides, bradycardia assumes a reflex character and develops as a cardiocardial reflex.

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THE RATE OF DEVELOPMENT OF IMMUNITY AFTER REVACCINATION WITH ANAEROBIC TOXOIDS

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A considerable volume of experimental and clinical evidence has now been accumulated on the rate of development of immunity after revaccination with tetanus toxoid. According to the majority of workers, an increase in the blood antitoxin titers is observed on the 3rd-5th day after revaccination. Separate workers assert, however, that after revaccination, the immunological reorganization of the body takes place much more quickly and an increase in the blood antibody titers is observed during the first 24 hours, or even within a few hours of injection of toxid into the animal [4, 5].

The study of the rate of development of immunity after revaccination is of great practical interest in solving the problem of finding a possible substitute for the prophylactic use of serum to prevent toxic infections in those previously immunized, in the form of revaccination with toxoids, for these preparations are more readily available and cheaper than therapeutic sera. Many workers have indicated that this replacement is possible in the prophylaxis of tetanus [1-3, 5-8]. In the U.S. Army, wounded who have previously been immunized against tetanus are given tetanus toxoid alone for prophylactic purposes [9].

In order to discover the more general character of development of antitoxic immunity after revaccination, in our research we used perfringens, oedematiens, botulinus toxoids in addition to tetanus toxoid.

EXPERIMENTAL METHOD

The investigations were carried out on animals and also on volunteer members of our staff, and these were given primary immunization with concentrated toxoids adsorbed on aluminum hydroxide.

In the first stage we studied the rate of increase of the blood antitoxin titers after revaccination in 12 human subjects. Experiments were also conducted on 38 rabbits and 3 monkeys (Macacus rhesus). Before revaccination and at various times afterwards, starting at 24 hours, the blood concentrations of perfringens, oedematiens, tetanus and botulinus type A antitoxins were determined. The sera were titrated individually by the usual method in white mice. To exclude variations in the titers of the sera due to the method of titration, the blood antitoxin titers in the same animal at different times were determined in the same titration experiment and using the same sample of standard toxin and the same batch of mice.

The animals used in the experiments had received different forms of preliminary immunological preparation (immunized once or twice). We studied the effect of primary and revaccination with different doses of toxids (from 0.1 to 5 ml).

EXPERIMENTAL RESULTS

In all the experiments the pattern of the results was the same for the various antigens: an increase in the blood antitoxin titers was observed not sooner than 3-5 days after revaccination. An increase in the doses of toxoid, replacement of adsorbed preparations by nonadsorbed and the creation of improved condictions for absorption of the toxoid (intramuscular injection) had no essential effect on the rate of increase of the blood

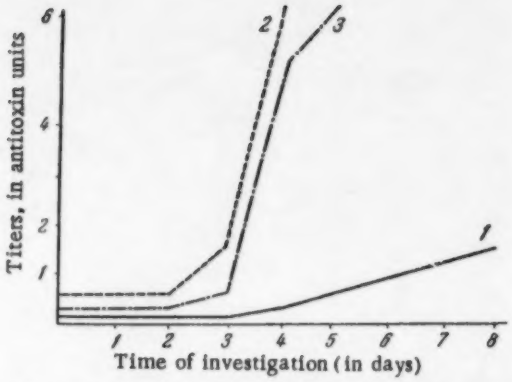

Fig. 1. Increase in the blood titers of perfringens, oedematiens and botulinus type A antitoxins in rabbits after revaccination. 1) Perfringens; 2) oedematiens; 3) botulinus type A.

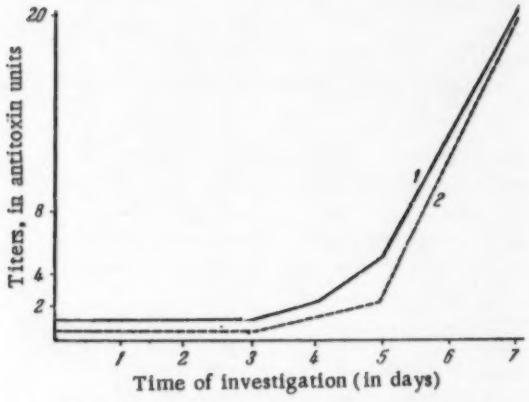

Fig. 2. Increase in the blood titers of tetanus and botulinus type A antitoxins in monkeys after revaccination. 1) Tetanus; 2) botulinus type A.

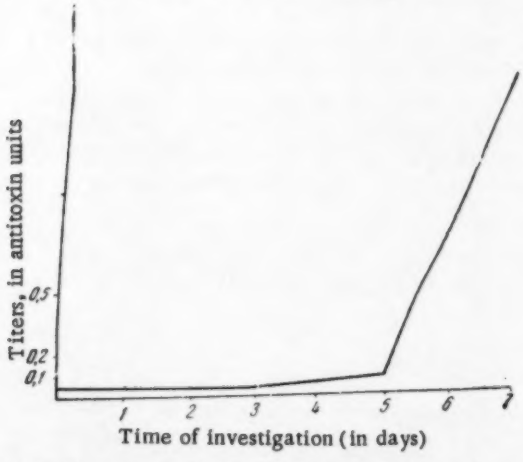

Fig. 3. Increase in the blood titers of tetanus antitoxin in human subjects after revaccination.

antitoxin titers. The experimental results are shown in Figs. 1-3.

It is not only the concentration of antibodies in the blood that determines the state of immunity; besides humoral factors, an important role is also played by tissue insusceptibility. Many workers have established that an increase in resistance after immunization may develop sooner than antibodies can be detected in the blood of the animals.

In view of this, in the next experiments, besides investigating the blood antibody concentrations, we studied the resistance of the animals to toxin at different times after revaccination. Experiments were carried out on rabbits, using perfringens and botulinus type A toxoids and toxins.

The results of the experiments showed that a clear increase in the resistance of the animals to toxin was observed 4-6 days after revaccination, parallel with a considerable rise in the blood antitoxin concentration. Two days after revaccination, no increase in the blood antitoxin titers could be detected by the ordinary methods of titration, but some increase in resistance was observed at this time, as shown by the survival of a larger number of experimental than control animals and by the less severe form of toxic manifestations in the experimental rabbits. Revaccination done at the same time as the toxin was injected has no prophylactic effect: the experimental animals died at the same times as the nonrevaccinated controls. The use of highly concentrated toxoids, increase in their dosage or fractionated injection of the preparations did not increase the effectiveness of revaccination.

Altogether we carried out 7 experiments on 216 rabbits. Similar patterns were obtained in all the experiments.

The increase in the resistance of the animals to toxin after revaccination shows itself only 1-2 days before the increase in the blood antibody concentration is found, and not less than 2 days after revaccination. The results of 2 experiments are shown in the table.

Our results are in agreement with the findings of the majority of workers on the relatively late development of immunity after revaccination (3-5 days). Our investigations did not confirm findings that a very early increase in the blood antitoxin titers takes place after revaccination [4, 5].

Revaccination, as a method of urgent prophylaxis, may evidently be used successfully to replace administration of antitoxic serum when the incubation period of disease is sufficiently long (tetanus). The rapid

Rate of Immunological Reorganization after Revaccination of Rabbits with Perfringens and Botulinus Type A Toxoids

Vaccine preparation	Revaccination		Times of injec. of toxin after revaccination (in days)	Doses of toxin	Blood antitoxin titers		Experimental results		
	doses of toxoid (in ml)	times after primary immunization (in months)			before revaccination	at the moment of injec. of toxin	Total number of animals	developed the disease	died
Perfringens toxoid	0,7—5	4	6	1 MLD	<0,1	0,5—1	5	1	0
			4			0,2—1	10	6	2
			2			<0,1	5	5	1
			Simultaneously with revaccination			<0,1	5	5	3
						<0,1	5	5	3
	Toxin control								
Botulinus type A toxoid	0,1—5	6	4	Minimal dose to overcome the immunity: 10 MLD for fresh animals	<0,025	0,05—0,25	8	0	0
			2			<0,025	15	14	7
			Simultaneously with revaccination			<0,025	5	5	4
						<0,025	5	5	4
	Toxin control								

prophylactic effect of revaccination is very unreliable in gas gangrene, in view of the short incubation period of this disease.

SUMMARY

Investigations conducted on volunteers as well as on rabbits and monkeys demonstrated that following revaccination with anaerobic toxoids (perfringens, oedematiens, tetanus, botulinus) the rise of the antitoxin titers is noted in the blood not earlier than on the 3rd-5th day.

An increased resistance to toxins occurs in the revaccinated animals 24-28 hours before the detectable rise of the antibody titer, but is noted only on the 2nd-3rd day after revaccination.

Replacing the administration of antitoxic serum by revaccination as a method of quick prophylaxis may succed only when the disease has a sufficiently long incubation period.

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INVESTIGATION OF THE FREE FORMALDEHYDE CONTENT WHEN POLIOMYELITIS VIRUS IS INACTIVATED WITH FORMALIN

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The considerable interest in the inactivation of viruses with formalin is due primarily to the preparation of antipoliomyelitis vaccine. Of the more important conditions of the inactivation process [4] which have not been considered quantitatively, there has remained the quantity of formaldehyde reacting with the virus. It is known that not all the formaldehyde added reacts with the virus, but that part of it is fixed by other components of the virus-containing fluid — amino acids, peptides and proteins — which pass into it from the synthetic medium 199 (Parker) and accumulate in the culture tissue in the process of proliferation. This bond is largely reversible.

In order to judge the quantity of formaldehyde capable of causing inactivation without essential depression of antigenicity, two values are therefore required — the total and the free formaldehyde.

Until recently, however, there has been no sufficiently reliable quantitative method of estimation of free formaldehyde [2, 8]. The only known indication was that of Taylor and Moloney [7] that, from the results of one approximate diffusion experiment, the content of free formaldehyde in monovalent poliomyelitis vaccine was about 90 %.

The method which we have devised for the determination of free formaldehyde in the presence of labile amino acid-formaldehyde and protein-formaldehyde compounds has enabled us to obtain the necessary values for the solution of this problem.

EXPERIMENTAL METHOD

We investigated different, independently prepared batches of virus fluid, obtained from a culture of kidney tissue in synthetic medium 199 and inactivated in accordance with Salk's [4, 5] recommendations by the addition of 1: 4000 formalin, giving a final formaldehyde concentration of 0.1 g/l.

In each case a sample for testing was taken on the 12th day of storage of the inactivated fluid at a temperature of 37°, i.e., in the phase of completion of the reaction between formaldehyde and the components of the virus fluid. The amino-nitrogen was estimated before its partial combination with formaldehyde, in the original virus fluid without formalin. The method of estimation of free formaldehyde has been described previously by us [1], and we shall merely give here a brief account of the principle of the analysis.

The investigation was carried out in a modified Conway's dish, in which the base of the inner chamber was slightly raised so that its depth was 1.5-2.0 mm. The outer chamber was filled with the test solution. A small volume of water (0.3 ml), covering the base of the inner chamber in a thin film, absorbed the gas evolved from this solution. In a state of equilibrium (after 42 hours) a concentration of free formaldehyde was produced in the absorbing liquid in which the presence of labile formaldehyde compounds did not interfere with the subsequent estimation of the formaldehyde by a colorimetric method [6].

TABLE 1

Free Formaldehyde Content in Inactivated Virus Fluid

Serial No.	No. of virus fluid	Free formaldehyde (in g/l)	Serial No.	No. of virus fluid	Free formaldehyde (in g/l)
1	30	0,071	15	55	0,080
2	31	0,081	16	56	0,076
3	32	0,036	17	57	0,085
4	33	0,081	18	58	0,075
5	35	0,075	19	59	0,086
6	43	0,089	20	60	0,079
7	44	0,086	21	62	0,080
8	44	0,080	22	66	0,084
9	48	0,085	23	67	0,081
10	49	0,080	24	68	0,072
11	50	0,085	25	69	0,078
12	51	0,076	26	70	0,078
13	52	0,079	27	71	0,080
14	53	0,078	28	72	0,077

Mean – 0.080 ± 0.009 g/l, $\delta = 0.0044$, medium 199 + formalin 1:4000 – 0.086 g/l.

TABLE 2

Total Nitrogen Content of Inactivated Virus Fluid

Serial No.	No. of virus fluid	Total nitrogen (in mg/l)
1	31	147
2	35	147
3	43	159
4	44S	147
5	44Zh	151
6	48	140
7	49	149
8	50	140
9	51	152
10	52	150
11	53	151
12	55	135
13	56	143
14	57	140
Mean		147 ± 12 $\sigma = 6,1$
Medium 199		139

Note: The figure of 156 mg/l of total nitrogen in the virus fluid before addition of solutions of formaldehyde and acetic acid corresponds by calculation to the mean total nitrogen content in the monovalent vaccines – 147 mg/l.

TABLE 3

Amino-Nitrogen Content of the Virus Fluid

Serial No.	No. of virus fluid	Amino-nitrogen (in mg/l)
1	32	126
2	33	117
3	43	124
4	44S	127
5	44Zh	121
6	60	122
7	62	115
8	65	126
9	69	122
10	70	120
11	71	120
12	72	122
13	73	121
14	75	118
Mean		121 ± 6 $\sigma = 3,4$
Medium 199		116

Note: Amino-nitrogen content of medium 199, by calculation 112 mg/l.

The total formaldehyde was estimated by the Haiberger-Nitschmann [3] method, the total nitrogen by Conway's method and the amino-nitrogen by the ninhydrin method.

EXPERIMENTAL RESULTS

It will be seen from the figures in Table 1 that the free formaldehyde content in the different batches of inactivated virus fluid was on the average 0.08 g/l. It is interesting that the medium 199 itself combined with almost tha same amount of formaldehyde, leaving 0.086 g/l uncombined.

It may be accepted that the formaldehyde in the virus fluid is combined mainly with the amino acids of medium 199, which do not undergo any essential quantitative changes in the process of cultivation of the virus (see also Tables 2 and 3).

Estimation of the total formaldehyde content of 5 batches immediately after addition of formalin showed that its value averaged 0.098 g/l (0.096-0.101 g/l), and in 20 batches at the end of the process of inactivation its value was 0.097 g/l (0.091-0.107 g/l).

The free formaldehyde thus accounted for 82.5 % of the total. The considerrable surplus of free formaldehyde reduced to a minimum the effect of small variations arising from treatment of the virus fluid, and ensured correct reproduction of its concentration.

In view of the practical importance of reproduction of the concentration of free formaldehyde, it appeared desirable to verify this conclusion by comparison with those chemical indices which reflect the concentration of those substances in the virus fluid that react with formaldehyde. The total concentration of these substances was judged by the total nitrogen present in the monovalent vaccines, and of the individual functional groups we investigated the most important — the amino group.

It may be seen from the figures in Tables 2 and 3 that the concentrations of the substances fixing formaldehyde were equally stable as the free formaldehyde. Hence it follows, in particular, that the practical recommendations directed towards the compensation of the supposed instability of the free formaldehyde concentration, and the resulting absence of standardization of the process of inactivation, for example the addition of glycine [2, 8] or further addition of formalin, have no sound basis.

We were thus able to establish that during the inactivation of the virus of poliomyelitis with formalin in accordance with the accepted recommendations [4,5] for preparation of the vaccine, the free formaldehyde content in the inactivated virus fluid had an average value of 0.08 g/l, and this concentration was readily reproduced in practice.

SUMMARY

The quantitative diffusion method of determihation of free formaldehyde has established that, inactivating poliomyelitis virus by Salk's method, the average free formaldehyde level at the end of the process was 0.08 g/l.

Formaldehyde in the virus fluid is combined mainly with amino acids passing into the fluid from the 199 medium. The free formaldehyde concentration is an easily controlled condition of inactivation.

This is confirmed by the stability of the chemical indices, the level of total nitrogen and amino nitrogen, reflecting the level of the substances interacting with formaldehyde in the virus fluid.

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ONCOLOGY

THE EFFECT OF VARIOUS DOSES OF IONIZING RADIATION ON THE ANTIGENIC AND BIOLOGICAL PROPERTIES OF BROWN-PEARCE CARCINOMA

COMMUNICATION II. CHANGES IN THE BIOLOGICAL PROPERTIES OF THE TUMOR

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(Received November 28, 1958. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

The subject of biological variation in malignant tumors by the influence of ionizing radiation has received insufficient study. In work by Goldfeder [3] it was shown to be possible to change the biological properties of mouse sarcoma 180 by irradiating it in vitro with a dose of 800-2000 r. The results of our previous research [1] demonstrate changes in the biological properties of the ascitic cells of Ehrlich's carcinoma by the action of x-rays on them in vitro.

The aim of the present work was to examine the effect of ionizing radiation, and in particular of x-rays, on the biological properties of the cells of the Brown-Pearce tumor of rabbits.

EXPERIMENTAL METHOD

A suspension of tumor cells from a Brown-Pearce carcinoma was irradiated in vitro with x-rays in doses of 2000, 10,000 and 20,000 r. The method of preparation and the conditions of the tumor suspension were described in detail in our first communication [2].

The experiments were performed on 4 groups of sexually mature male chinchilla rabbits of the same weight and age, with 5 animals in each group.

All the animals were inoculated at the same time intratesticularly with 0.5 ml of a suspension of Brown-Pearce carcinoma cells. The first group of rabbits was injected with a cell suspension irradiated with 2000r, the second — 10,000 r, the third — 20,000 r, and the fourth or control group were given the same volume of a 20% suspension of untreated tumor cells.

EXPERIMENTAL RESULTS

Observations on the inoculated animals showed differences in the clinical appearance of the tumor in the experimental and control groups of rabbits. In the table are given the combined results for each group, showing the duration of survival of the inoculated animals, the times of appearance of the tumor in them, and the numbers of rabbits which died or survived.

It will be seen from the results in the table that the shortest latent period — 4.2 days — was observed in the

The Influence of Various Doses of Irradiation on the Clinical Course of the Tumor

Group of animals	Animal No.	Latent period (in days)	Mean laten period (in days)	Number of rabbits dying	Number of rabbits surviving	Duration of life of rabbits which died (in days)	Mean duration of life of rabbits (in days)
1st-irradiated with 2000 r	1667	6	7.0	2	3		
	2153	8					
	3222	7					
	3259	5				75	56,5
	3967	9				38	
2nd-irradiated with 10.000 r	1485	4	5,6	4	1		51,5
	2384	5				45	
	3186	7				52	
	3426	7				69	
	3981	5				40	
3rd-irradiated with 20.000 r	3595	5	6,4	5	0	18*	48,2
	3610	6				29	
	3792	7				47	
	3926	8				55	
	2362	6				62	
4th-controls	2382	3	4,2	5	0	30	30,0
	2383	5				28	
	2405	4				27	
	3446	4				24	
	3907	5				41	

* Rabbit No. 3595 died from accidental causes on the 18th day and was not taken into consideration when the mean duration of life of the group was calculated.

Note: In rabbits Nos. 1667, 2153, 3222 and 1485 the tumors were absorbed and the animals are still alive (13 months later).

control group of rabbits. In the animals inoculated with irradiated carcinoma tissue, the latent period was lengthened to 5-7 days. All the animals of the control group died from the tumors, which developed extensive metastases. Their mean duration of survival was 30 days.

In the animals inoculated with irradiated Brown-Pearce carcinoma cells, the tumor was observed to follow a different course, which was evidently related to the inequality of the doses of irradiation.

For instance, the rabbits of the 3rd experimental group, inoculated with cells irradiated with 20,000 r, died from metastases, but their mean duration of life was almost twice that of the control animals, being 51.2 days. In the 2nd experimental group of rabbits, inoculated with tumor tissue irradiated with 10,000 r, four of the rabbits died with obvious tumor metastases, and in one the tumor was absorbed on the 22nd day after inoculation. The mean duration of life of the rabbits in this group which died was 51,5 days. Of the rabbits inoculated with cells irradiated with 2000 r, two died, and in three animals the tumors were completely absorbed after a period of growth.

Statistical analysis showed that the differences in the mean indices of the latent periods of the 1st and 4th, and also of the 3rd and 4th groups were significant (P=0.008). The differences between the 2nd and 4th groups were not significant (P=0.08). The differences between the mean durations of life of the 2nd and 4th groups of animals were statistically significant (P=0.01). Analysis of the results for the 3rd and 4th groups showed that the differences between them were close to significant (P=0.03). The significance of the differences between the 1st and 4th groups is undoubted.

The atypical development of the tumor in rabbits inoculated with irradiated material is very characteristic. It was shown by the fact that when the tumor reached a certain stage of development , for example the size of a pea, it decreased in size, showed obvious regression, then increased in size once more and led to the death of the animal .

The appearance of atypical development of the tumor in the experimental groups of animals varied in accordance with the dose of x-rays used to irradiate the original material.

For instance, tumor cells irradiated in vitro with large doses of x-rays (in our experiments 20,000 r), when inoculated intratesticularly, although they caused prolongation of the latent period and of the duration of life of the rabbits, and also temporary partial regression of the palpable tumor, nevertheless led eventually to the death of the animals. The atypical course of development of the tumor in the group of animals inoculated with cells irradiated with 10,000 r hardly differed from that described above.

Tumor cells irradiated with comparatively small doses of x-rays (in our experiments 2000 r), when inoculated intratesticularly , showed a more marked atypical course of the tumor , accompanied in the majority of cases by repeated and considerable regressions of the tumor, even by complete absorption and clinical recovery of the experimental animals. In cases of complete absorption of the tumor (rabbits Nos. 1667, 2153, 3222) , a lasting immunity to further inoculation of tumor tissue was observed.

The results obtained demonstrate that the action of x-rays on Brown-Pearce carcinoma cells in vitro causes a change in the biological properties of the tumor cells, and moreover that the intensity of the change shows a definite relationship to the dose of irradiation. In contrast to doses of 20,000 r, in our experiments doses of 2000 r gave the most intensive changes in the biological properties of the tumor cells.

SUMMARY

If intratesticular tissue of Brown-Pearce carcinoma is inoculated in rabbits following irradiation by x-rays in vitro (2000—20 000 r) the biological properties of the tumor cells are altered, the latent period of the tumor development prolonged, the life span increased , and atypical development, and in some cases, even complete resolution of the tumor are observed.

The intensity of the mentioned changes depends directly upon the dose of irradiation.

As compared with a dose of 20,000 r the changes in the biological properties of the tumor cells after irradiation with 2000 r were found to be more pronounced.

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INVESTIGATION OF THE ACTION OF SIX ALKALOIDS DERIVED FROM 1-METHYLPYRROLIZIDINE ON THE GROWTH OF HEPATOMA AND OTHER TRANSPLANTED ANIMAL TUMORS

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Compounds with a well-marked characteristic action on certain tissues of the body are of undoubted interest for investigation of their possible action also on tumors arising from these tissues.

Plant alkaloids of the genus Senecio, Heliotropium and certain others are derivatives of 1-methylpyrrolizidine, and are complex esters, splitting up on saponification into aminoglycols (heliotridin, retronecin, platynecin, etc.) or amino alcohols (for example trachelantemidine) and acids (heliotrinic, lasiocarpinic, senecioninic, viridiflorinic, etc.). The alkaloids of this group are known to possess marked hepatotoxic properties [10-12] and, in addition, some of these alkaloids, when introduced into the body over a long enough period of time, cause the development of tumors in the liver of animals [13-15]. According to some findings, chronic alkaloid poisoning is associated with changes in the metabolism of the liver cell, leading to an increase in the concentration of copper in the liver. This is shown pathohistologically mainly by an increase in the size of the liver cells and their nuclei, and by a decrease in the regenerating power of the liver tissue, although in isolated cases foci of hyperphasia may also be observed [10, 11, 13].

We investigated the action on a hepatoma transplanted subcutaneously into mice of line C_3HA of six alkaloids, the chemical structure and properties of which have been described in the literature (heliotrine [4], viridiflorine [6-8], platyphilline [3, 9], seneciphilline [3, 9], lasiocarpine [4, 5] and heliosupine [2]). The strain of transplantable hepatoma which we used was obtained and described by V. I. Gel'shtein [1]. At the same time, we investigated the action of these same alkaloids on the growth of an Ehrlich's tumor, grafted subcutaneously and in some cases, on the growth of sarcoma 45 of rats.

EXPERIMENTAL METHOD AND RESULTS

The drugs were injected intraperitoneally once a day. Heliotrine was used in its basic form, platyphillin as the bitartrate, and the remaining 4 alkaloids as the hydrochloride.

As shown by the results of an investigation of the toxicity, given in the table, the most toxic were seneciphillin, lasiocarpine and heliosupine, whereas the other three alkaloids were less toxic. From a comparison

Results of an Investigation of the Toxicity and the Inhibitory Action on the Growth of Tumors of the Alkaloids Investigated

Experiment No.	Preparation	100% lethal dose, mg/kg	Therapeutic dose, mg/kg	Tumor	Day after transplantation when expt. started	Duration of treatment, in days	Number of animals: experiment	Number of animals: control	Inhibition of tumor growth by comparison with controls in wt. %
1	Heliotrine	400 (for mice)	40	Hepatoma	8	14	10	10	8
2	»		100	Ehrlich's tumor	5	12	10	10	0
3	»	300 (for rats)	20	Sarcoma 45	7	14	15	15	0
4	Viridiflorine	500 (for mice)	100	Hepatoma	8	14	10	10	0
5	The same		100	Ehrlich's tumor	5	12	10	10	0
6	Platyphillin	400 (for mice)	100	Hepatoma	8	14	10	10	0
7	The same		120	Ehrlich's tumor	5	12	10	10	0
8	Seneciphillin	120 (for mice)	20	Hepatoma	8	14	10	10	10
9	The same		20	Ehrlich's tumor	5	12	10	10	19
10	Lasiocarpine	300 (for mice)	20	Hepatoma	9	14	10	10	21
11	»		30	Ehrlich's tumor	5	12	10	10	0
12	»	100 (for rats)	10	Sarcoma 45	7	14	15	15	17
13	Heliosupine	150 (for mice)	40	Hepatoma	8	14	14	14	0
14	»		50	Ehrlich's tumor	5	12	10	10	54
15	»	100 (for rats)	15	Sarcoma 45	7	14	10	10	28

of the values of the lethal dose ($LD_{100}\%$) of heliotrine, lasiocarpine and heliosupine, determined in mice and rats it followed that rats were more sensitive to the toxic action of these compounds than mice. Approximately the same relationships held good for the therapeutic doses for rats and mice.

It was found that mice of the C_3 HA line with hepatoma were slightly more susceptible to the toxic action of these drugs when given repeatedly, than mongrel mice with Ehrlich's tumor.

When the alkaloids were given in the therapeutic doses shown in the table, none of the animals under observation died in the course of the 12-14 days of the experiment, but after its completion the majority of the mice showed dystrophic changes, as mentioned above, in the liver in agreement with the findings of other authors [10-13].

The only exception was viridiflorine, for after administration of this drug the liver was normal in appearance. In this connection it must be remembered that viridiflorin is a complex ester, not of an amino glycol, like the rest of the alkaloids under test, but of an amino-alcohol — trachelantamidine. This difference in its structure evidently accounts for the difference in its action on the liver. However,

notwithstanding the toxic action of nearly all the alkaloids investigated on the liver only one of them (lasiocarpine) had a slight inhibitory action on the growth of the hepatoma (see table).

Investigation of the action of the same compounds on the growth of the Ehrlich's tumor showed that only heliosupine give a significant inhibitory effect (54 % inhibition by comparison with the controls); repetition of the experiment gave the same result. Seneciphillin inhibited growth of this tumor only to a slight degree (19%), and the remaining compounds tested had no action on its growth. A weak inhibitory action on the growth of sarcoma 45 was shown by lasiocarpine and heliosupine.

As a result it can be stated that, although three of the compounds investigated — seneciphillin, lasiocarpine and heliosupine — possess slight antitumor activity, this does not agree, as we thought at first, with the toxicological organotropy of the compounds of this group, since they do not exhibit their main action on the hepatoma, even in the case of heliosupine, when the antitumor activity which is present is expressed as a considerable inhibition of the growth of Ehrlich's tumor.

The pronounced toxic action of these compounds on the normally functioning liver and their lack of action on malignant tissue give grounds for suggesting that in this case the toxic action of the drugs is connected with a function of the normal liver that is absent from tumor cells derived from the tissue of that organ. In such cases, toxicological organ-specificity naturally provides no clue in the search for antitumor preparations.

SUMMARY

Investigations were made to determine the toxicity and the effect of 6 alkaloids (heliotrine, viridiflorin, platyphillin, seneciphilline, lasiocarpine and heliosupine) on the growth of subcutaneously inoculated hepatoma of mice and Ehrlich's adenocarcinoma. The greatest inhibitory effect on the growth of the hepatoma was shown by lasiocarpine. A considerable inhibitory effect on Ehrlich's tumors was also exerted by heliosupine.

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EXPERIMENTAL BIOLOGY

THE EFFECT OF RADIOACTIVE RADIATION OF VARIOUS STRENGTHS FROM Co^{60} ON THE REGENERATING POWER OF SKELETAL MUSCLE

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In the present research we studied the effect of total, prolonged exposure to γ-radiation from Co^{60} on the processes of regeneration in striped muscle, using different doses of irradiation, and different distributions of the same dose in time, and compared this with the effect of a single total exposure of short duration, as described by us in an earlier paper [4]. So far as we know, no research has been done on this subject.

EXPERIMENTAL METHOD

Experiments were carried out on male white mice of the same age and weight. More than 350 animals were used, and were kept on the same diet. The test object was the tibialis anterior muscle, regenerating after mechanical trauma. For this purpose an incision was made through the skin and halfway through the middle of the muscle in its depth. The course of the process of regeneration was examined at the following times: 12, 24 and 48 hours, and 3, 5, 8, 10, 12, 15, 17, 20, 25 and 30 days after infliction of the trauma. The material was fixed in Zenker-formol; the whole of the tibialis anterior muscle was embedded in celloidin-paraffin wax. Serial sections were stained with Carazzi's hematoxylin and with Mallory's eosin, with Heidenhain's iron-hematoxylin, with azocarmine and with azure II-eosin.

In all, 8 series of experiments were carried out in which animals were exposed to total, prolonged, continuous irradiation. Two groups of mice acted as controls and were not irradiated. In addition, for comparison, we used animals irradiated for short periods of time, exposed to a single total irradiation with γ-radiation from Co^{60} in a GUT-400 telecobalt apparatus, in doses of 250, 500, 800, 1000 and 1500 r, and at a dose rate of 21 r/min.

The total, prolonged irradiation of the animals was carried out in a special room, in which was a stable telecobalt apparatus which was in operation 24 hours a day. The range of dose rates used was from 0.003 to 0.07 r/min. In order to obtain the desired dose rate, the animals were placed at different distances from the source of radiation.

In these series of experiments, the posttraumatic regeneration was studied after a total dose of 800 r, given over a period of 8 days, $1\frac{1}{2}$ months and 6 months of continuous irradiation, and after a dose of 1500 r, given over periods of 15 days, $1\frac{1}{2}$ months and 6 months. Two series of experiments comprised animals which were given a dose of 2300 r over periods of 5 months and $10\frac{1}{2}$ months of continuous, prolonged and total irradiation. In all cases the trauma was inflicted on the day following cessation of the irradiation. The control animals underwent operation at the same time.

EXPERIMENTAL RESULTS

All the irradiated animals developed radiation sickness, the severity of which depended on the dose and also on its intensity. The character of the radiation sickness was assessed by the peripheral blood picture, the weight of the spleen and the general condition of the animals.

Microscopic analysis of the material showed that in the control animals, after mechanical trauma, destructive changes developed in the striped muscle fibers immediately exposed to injury, which were expressed, primarily, as disappearance of the transverse and longitudinal striation, lumpy and granular destruction of the sarcoplasm and death of the muscle nuclei. The degenerative changes spread from the focus of direct injury for a certain distance in either direction. The areas of muscle fibers suffering destruction were cleared by lysis and phagocytosis by the blood connective cells. On the 3rd-4th day, resorption of the necrotic masses was mainly completed.

Side by side with these processes of active degeneration and resorption, on the 2nd day changes of a regenerative character were found in the injured muscle. Fibroblasts undergoing mitotic division were seen in considerable numbers, which were especially large on the 4th-5th day. At the ends of the areas of viable muscle fibers there was an accumulation of basophilic sarcoplasm, which stained a light or dark blue color with azure II-eosin. These areas of basophilic sarcoplasm later were the sources of muscle buds and myosymplasts. The latter were formed not only from growth of muscle buds but also by cleavage of the basophilic sarcoplasm along the length of the fiber in the form of myosymplastic bands with chains of nuclei.

On the 5th day the defect was filled with granulation tissue and myosymplasts, in which later appeared a myofibrillary system, so that they became converted into muscle tubes. On the 8th day cartain muscle tubes were already differentiated into young muscle fibers (see Figure 1, a).

The process of regeneration of the skeletal muscle from the 8th-10th day to the 30th day after mechanical trauma was simply the further differentiation of muscle tissue. On the 30th day a muscle and connective tissue area of regeneration was formed, consisting of adult muscle fibers with a well-marked cross striation and sparsely arranged nuclei beneath the sarcolemma; between the fibers were seen small connective tissue septa. This area of regeneration differed from normal muscle tissue only by the irregular arrangement of its muscle fibers (see Figure 3, a).

We have previously reported [4] that in uninjured muscle, after single, total irradiation, insignificant morphological changes were from time to time observed. The muscle tissue of animals exposed to prolonged irradiation showed absolutely no morphological changes. Under these circumstances, however, it was found that the muscle had suffered considerable radiation injury, which became apparent only during posttraumatic regeneration, the course of which was significantly depressed by comparison with the controls.

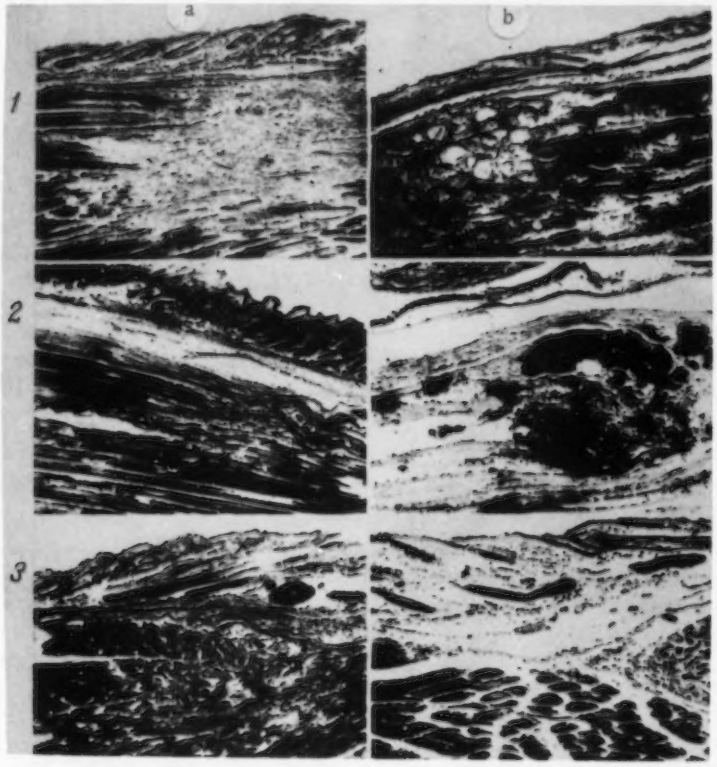
As a result of chronic irradiation of animals with a dose of 800 r, the exposure to radiation continuing uninterruptedly for 8 days and 1½ months, an appreciable depression of the process of regeneration of muscle tissue was observed; it was especially noteworthy in the experiments in which mice were irradiated with a dose of 1500 r, extending over periods of 15 days and 1½ months.

Detailed histological analysis showed that with a total dose of 1500 r, given over a period of 15 days, in the first stages of regeneration the process was indistinguishable from that observed after a single, total irradiation with the same dose. In either case it was shown as a sharp depression of the leucocytic reaction and of the reaction of the connective tissue cells and as very extensive destructive changes throughout almost the whole extent of the divided part of the muscle, especially at its distal end. Destructive processes were predominant in the region of the wound for a long time, and the regenerative processes were sharply depressed. Whereas in the control animals, at the end of the second day the involvement of new areas of muscle fibers in the destructive process was already restricted, in the case of animals irradiated for 15 days, destruction at a distance from the immediate area of trauma continued until the 8th or even the 10th day.

On account of the depression of the leucocytic reaction, of the diminished lysing function of the microphages, and also the lowered phagocytic activity of the macrophages, even on the 8th day resorption of the necrotic masses from the wound was completely absent, as after a single, total exposure of short duration to irradiation (see Figure 1, b). At the site of the defect, in contrast to the controls, lay untouched necrotic masses, occasionally a large quantity of effused blood and fibrin threads. Excessive and prolonged edema of the tissues was also observed.

Even on the 8th-10th day after total chronic irradiation, there were still hardly any signs of the appearance of regeneration. The solitary buds developing at this time were often defective and underwent regeneration; in the region of the defect, as a rule, no muscle tubes were found, and often even no muscle buds.

The proliferative power of the fibroblasts, and also their collagen-forming function, were depressed to an equal degree. Whereas in the control animals, on staining by Mallory's method, slender collagen fibrils could be seen on the 4th-5th day, in animals irradiated for 15 days, just as in those given a single irradiation of the same dose (1500 r), hardly any collagen fibrils were to be seen on the 8th day. In the controls, on the 2nd-3rd day after trauma, a large number of fibroblasts undergoing mitotic division could be seen, but in the experiments with irradiation for 15 days, and also after a single irradiation, no mitotically dividing cells could be observed in the films on the 5th nor on the 8th-10th days; under these circumstances large numbers of giant fibroblasts and binuclear cells were encountered, which had previously been observed by other authors after local irradiation with x-rays [1, 3, 6].



The state of the regenerating muscle tissue in mice exposed to irradiation and in control animals. 1,a) Control, 8th day after trauma; 1,b) total, chronic irradiation for 15 days; dose — 1500r, 8th day after trauma; 2,a) control, 15th day after trauma; 2,b) total chronic irradiation for 15 days, dose 1500 r, 15th day after trauma; 3,a) control, 30th day after trauma; 3,b) total chronic irradiation for 5 months, dose 2300r, 30th day after trauma. Microphotographs. Magnification: ocular 5×, objetive 8×.

Comparison of the findings shows that the depression of the process of regeneration in skeletal muscle after single irradiation of short exposure and irradiation of mice for 15 days with radiation from Co^{60} in a dose of 1500 r was very similar, despite the fact that the general effects on the body differed sharply in the two cases. Whereas with single, total irradiation of short exposure, in a dose of 1500 r, all the mice had died by the 9th-10th day, after total irradiation for 15 days the animals survived for 30 days. This permitted the histological changes

to be observed at later periods. It could be seen that even on the 15th day the necrotic masses were still unabsorbed, and remained in large quantities in the region of the defect (see Figure 2,b). Naturally these acted as an obstable to the completion of regeneration of the skeletal muscle.

It is interesting that at this time a considerable number of myosymplasts was observed in the distal end of the injured area of the muscle, at the periphery of the defect, whereas at the proximal end necrotic masses of muscle tissue remained, as it were, static, like the appearance on the 15th day after a single total exposure to a dose of 1000 r. No signs of regeneration of muscle fibers could yet be seen at the proximal end. In the controls, on the 15th day, as a rule the region of the defect was filled with young muscle fibers, with connective tissue septa between them (see Figure 2,a). On the 30th day, no necrotic masses could be seen in the irradiated animals of this series, and the region of the defect was filled mainly with fatty tissue, with undifferentiated muscle cells scattered here and there.

In the experiments in which a dose of 1500 r was attained in $1\frac{1}{2}$ months, the process of regeneration of muscle tissue was also appreciably depressed. The young muscle cells which appeared were retarded in their growth by comparison with the controls, and did not always reach the center of the wound. For example, on the 8th day, in contrast to the controls, only muscle buds and short myosymplasts were seen at the periphery of the defect, and in the center there remained remnants of necrotic masses and loose connective tissue (see Figure 1,b). The connective tissue and leucocytic reations were, however, depressed to a much less degree than in the previous series of experiments (1500 r over a period of 15 days). On the 30th day the differences in the course of the process of regeneration in these two series of experiments (1500 r over periods of 15 days and $1\frac{1}{2}$ months) were not very pronounced.

In the chronic irradiation experiments, in which the same total dose (1500 r) was given over a period of 6 months, some signs of depression of regeneration of the muscle tissue were also observed, but to a much less degree.

In the next series of experiments with chronic, continuous γ-ray irradiation we used a total dose of 2300 r. One group of animals received this dose over a period of 5 months, another over $10\frac{1}{2}$ months. The dose rate in the first case was almost double, and in the second case equal to that used in the preceding series of experiments (1500 r over a period of 6 months).

The depression of regeneration of muscle tissue by a dose of 2300 r was expressed to a far greater degree than that by a dose of 1500 r, given over a period of 6 months. This is all the more interesting because in the course of the inflammatory process, no essential differences were observed. In all these experiments (1500 r over a period of 6 months, 2300 r over 5 months and 2300 r over $10\frac{1}{2}$ months) the leucocytic reaction and the reaction of the connective tissue cells were depressed to a considerably less extent than in the series in which the irradiation was given as a single exposure or spread over 8 and 15 days. The intensity of spread of the regeneration of muscle fibers away from the zone of direct injury was hardly indistinguishable from that of the controls; there was no delay in the liquidation of necrotic masses from the wound. The proliferative power of the fibroblasts, and also their collagen-forming function, were depressed to a less extent.

Despite these favorable circumstances, which distinguished the course of the process of regeneration essentially from that observed after a single exposure or after irradiation lasting 8 or 15 days, a total dose of 2300 r, whether given over a period of 5 months or $10\frac{1}{2}$ months, perceptibly depressed the regenerative power of the muscle tissue. Muscle cells, appearing in the course of regeneration, sometimes had not reached the center of the defect even on the 30th day (see Figure 3,b).

We have previously mentioned that after a single irradiation of short exposure, when the same dose rate was used, the degree of depression of the process of posttraumatic regeneration of skeletal muscle depended on the dose and on the severity of the radiation sickness [4]. The most pronounced depression of regeneration was observed with γ-ray irradiation from Co^{60}, given as a single dose of 1500 r; with doses of 1000 r and 800r the degree of depression was proportionately reduced. Doses of 500 and 250 r caused no essential differences in the course of the process of regeneration by comparison with the controls; merely certain changes in the process of inflammation were observed, as has been pointed out also by other authors [2, 4, 6].

Our conclusion from an analysis of our findings is as follows. In the case of total, chronic, continuous irradiation of mice with γ-rays from Co^{60}, depression of the regenerative power of striped muscle tissue takes place. The degree of this depression is less expressed at early stages when the irradiation is spread over a long

period of time than when the same total dose of irradiation is given in a single exposure; this is due, it must be assumed, to the difference in the severity of the radiation sickness. The influence of the general condition of the animal on the course of the process of regeneration in the traumatized muscle is all the more likely because degeneration of the muscle fibers, distortion of the inflammatory process and delay in the resorption of the necrotic masses from the wound were much more pronounced after a single total exposure to irradiation than in the case of chronic exposure.

Not all these factor, naturally, could affect the process of regeneration of the muscle tissue , but they are not , however, the only cause of delay in and depression of regeneration.

After total irradiation of an animal, direct injury of muscle tissue also takes place , which is capable of lasting for a long period of time, as in the case of local irradiation os muscle [6] . In case of chronic irradiation, this is a latent radiation injury, appearing only during posttraumatic regeneration, and also capable of summation. The property of striped muscle to retain and to summate radiation damage is presumably explained, as G. S. Strelin believes, by the almost complete absense of power of physiological regeneration of this tissue, during which process recovery could take place [5].

SUMMARY

Experiments were performed on mice with total continuous (Co^{60}) irradiation of short and long duration. After discontinuance of the irradiation mechanical trauma was inflicted to the anterior tibial muscle. Regeneration in irradiated and control animals was studied in histological preparations. It was demonstrated that regeneration is completely inhibited by a single irradiation with 1500 r (the dose rate being 21 r/ min); the regenerative function , although considerably depressed, is retained with the doses of 1000 r and 800 r, and hardly differs from the control, with the dose of 500 and 250 r.

In chronic irradiation (with the dose rate of from 0.003 to 0.07 r/ min), only the dose of 800 r, administered within 6 months, did not alter the course of regeneration in comparison with controls. In all the remaining experiments (800 r for the period of 1-5 months and 6 months , 1500 r for the period of 15 days , 1½ months and 6 months ; 2300 r for the period of 5 and 10½ months) a clear-cut depression of the regenerative power of the muscle tissue could be observed, which rose along with an increase in the dose administered.

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THE STRUCTURE OF THE REGENERATING LIVER IN RATS

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Large-scale investigations have recently been carried out into the study of the regenerative powers of mammalian organs, and especially the regeneration of the internal organs. The heightened interest of research workers in this problem has been due to an attempt to find an experimental basis for new theoretical concepts, and to the requirements of practical medicine.

The great regenerative powers of certain internal organs of mammals are not now in doubt. Relatively little attention has been paid, however, to the problem of the structural and functional qualities of the regenerating organ, which requires more detailed analysis.

For instance, it has been shown that removal of a large part of the liver, up to four fifths of its mass, in mammals, very quickly leads, in 7-14 days, to complete restoration of the initial size of the organ. The removed lobes are not reformed under these circumstances, but a regenerative hypertrophy of the residual lobes of the liver takes place [3, 8, 10, 11, 12].

This rapid restoration of the damaged liver is brought about by the intensive mitotic proliferation of the liver cells. Proliferation of the cells of the efferent ducts and the stroma takes place to a lesser degree [9].

After extirpation of two lobes of the liver in rats, on the 3rd day the number of liver cells in the regenerating liver is 65%, and on the 12th day 89%, of the number of liver cells in animals not undergoing operation [6]. The question whether such a rapid quantitative replacement of the cells is accompanied by structural changes in the organ has received only little study.

In earlier work [3, 11] it was asserted that the microsoopic structure of the regenerating liver (after the surgical procedure described above) was characterized by hypertrophy of the hepatic lobules. These authors did not describe the formation of new structural units of the organ. Later workers, however, concerned with the study of regeneration of the liver, began to hold that the formation of new lobules does take place in the liver, and that these are indistinguishable from normal in their structure [4, 8].

These divergent views may be explained by the absence of special research directed toward the solution of this problem. Little attention has been paid, in particular, to the study of the topography of the blood vessels in the regenerating liver, which could give much valuable evidence about its structure.

We know only one paper [5] in which it is pointed out that the blood pressure in the portal vein of a regenerating liver is lowered, and thus, according to the views of Elias [6], the arrangement of the liver lobules is inverted in character, i.e., the portal and not the hepactic vein is in the center of each lobule.

This state of affairs impelled us to carry out the present investigation, with the special object of ascertaining the dimensions of the lobules in the regenerating liver of rats, by demonstrating the topography of the arterial vessels of the liver.

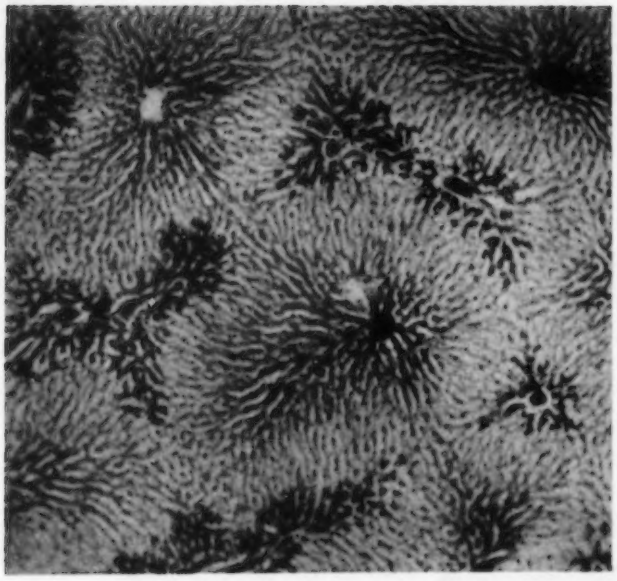

Fig. 1. Section of the normal liver of a rat after injection of the arterial vessels with India ink. The contours of the individual lobules are clearly seen. Magnification 75x .

Number of Central Veins in a Field of Vision and Area of the Lobule in the Liver of the Experimental and Control Rats

Group of animals	Indices used	Time of regeneration in days											
		1	2	3	7	12	14	20	30	50	75	90	180
Control	Number of central veins in a field of vision	8	—	8	—	8	9	—	—	8	—	9	9
	Magnified area of section of lobule, in mm²	147	—	127	—	153	141	—	—	164	—	169	178
Experiment	Number of central veins in a field of vision	7	6	5	4	4	4	5	5	4	5	5	4
	Magnified area of section of lobule, in mm²	193	231	386	373	397	340	293	343	416	358	371	383

EXPERIMENTAL METHOD

In male white rats, weighing 115–150 g, total extirpation of the left lateral and central lobe of the liver was performed , and ligatures applied to the bases of these lobes by the method of Higgins and Andersen. The portion removed accounted for 60–70 % of the weight of the organ. The regenerating liver was investigated 1, 2, 3, 7 , 12, 14, 20, 30, 50, 75, 90 and 180 days after operation. At each time 5 experimental and 3 control animals were sacrificed.

The main difficulty in demonstrating the strucgure of the regenerating liver was the absence of any clear division into lobules. The contours of the lobules could, however, be quite clearly distinguished by injection of a mixture of India ink and gelatin into the arterial system of the liver. A 4% solution of gelatin in ink, warmed to 37-40°, was injected into the anesthetized animal, through a thoracotomy directly into the aorta, in a volume of 1 cm^3. Before injection a first ligature was tied above the site of injection and after the injection a second ligature was tied below the site.

After injection of the ink the circulation must be stopped immediately, otherwise the ink escapes from the arterial capillaries into the venous, so that the contours of the lobule are obliterated. The sacrificed animal was placed without delay in the refrigerator for 30 minutes at a temperature of 0°, and was then kept for 24 hours at +2°. After 24 hours the liver was fixed in 10% formalin, and sections were cut to a thickness of 10μ on a freezing microtome.

The upper part of the right lobe of the liver was removed, not far from its base, from both experimental and control animals; this was the most convenient place for the preparation of tangential sections. The structure of the lobule was seen best of all in sections passing just beneath the capsule. In these areas nearly all the lobules were cut transversely, which facilitated their counting and measurement.

For measuring the area of the lobules sections of equal size were used, passing at roughly the same distance from the capsule. By means of an Edinger drawing machine, with magnification 19 times, the contours of all the lobules in one section from each liver were traced on cleaned x-ray film. The section usually contained 12-26 lobules. Areas of film, corresponding to the individual lobules, were cut out and weighed on torsion scales. The values obtained were divided by the weight of 1 cm^3 of film. Having estimated in this way the area lobule, the mean area of the lobules was then calculated.

In order to analyze the structural relationships between the vessels of the regenerating liver and the parenchyma, and also to provide supplementary information on the dimensions of the lobules, the number of central veins in a field of vision of the microscope was counted, at a magnification of 20 times. Three fields of vision were used in each section.

EXPERIMENTAL RESULTS

In the liver sections, the arterial capillaries situated around the periphery of the lobule were filled with injected ink and clearly outlined its contours. The central veins were well marked, thanks to their central position in the transverse sections of the lobules, and to the almost complete absence of dye from them (Fig. 1).

In the first few days after operation, dilatation of the central and peripheral veins of all the lobules, and also of their venous and arterial capillaries, was observed.

The results of the counts of the number of central veins in a field of vision of the microscope, and of the measurement of the area of the lobules in the section in the experimental and control animals, are shown in the table. For each period of regeneration the mean values obtained in three animals are given.

The results obtained show that the dimension of the hepatic lobules began to change quite early. Within 24 hours of operation, the dimensions of the hepatic lobules in the regenerating liver were increased. The number of central veins in a field of vision in the liver preparations from the control animals was 8, and from the experimental animals 7. The corresponding areas of the lobules were 147 and 193 mm^2. The differences between the experimental and control values were statistically significant. The increase in size of the liver lobules in the first 24 hours was evidently due both to an increase in the size of the hepatic cells, as pointed out by several authors [3, 10, 11], and to considerable dilatation of the blood vessels.

On the second day, when proliferation of hepatic cells began in the partially resected liver, the increase in the size of the liver lobules took place more intensively. The number of central veins in a field of vision was 6, the area of the lobule 231 mm^2. On the third day after operation, all the liver lobules were grossly hypertrophied. One field of vision included 5 central veins, whereas in the liver sections from the control animals there were 8 as before. At this time the area of the liver lobule in the experimental animals was 386, and in the controls 127 mm^2.

The difference in the size of the lobules on the second and third days after operation was statistically significant. Thus the dimensions of the liver lobules in the regenerating liver were roughly 2-3 times as great

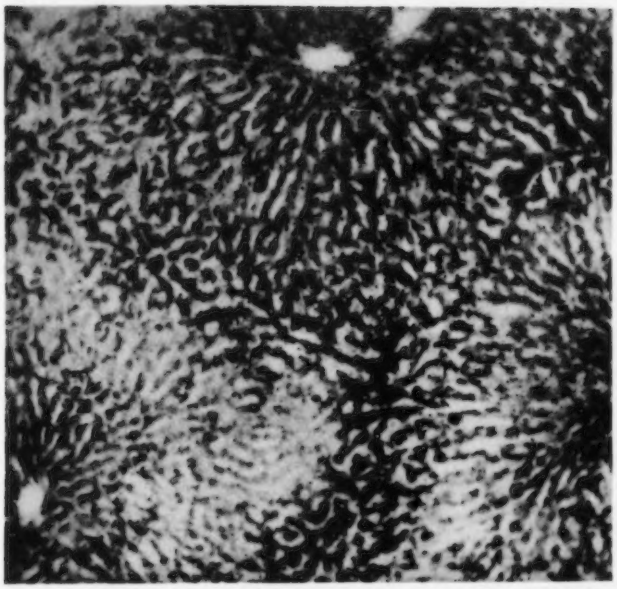

Fig. 2. Section through the regenerating liver 3 days after partial hepatectomy. Dilated arterial capillaries and veins are seen. The hepatic lobules are hypertrophied. Magnification 75 x.

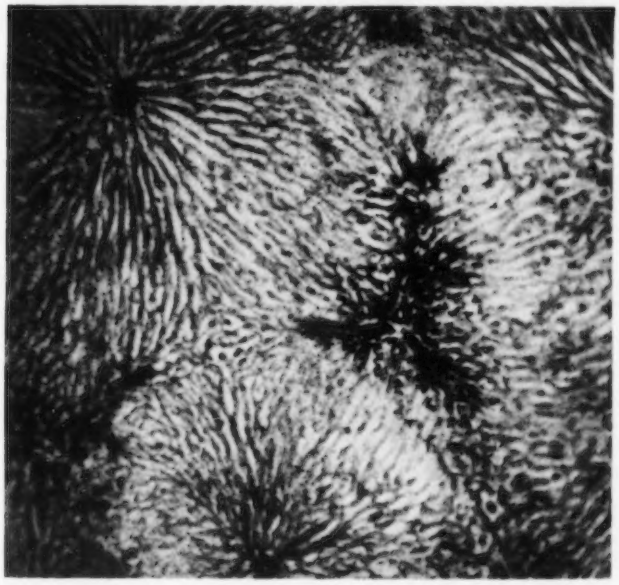

Fig. 3. Section through the regenerating liver 6 months after hepatectomy. Normalization of the filling of the blood vessels. Liver nodules hypertrophied. Magnification 75 x.

as those of the control animals on only the third day (Fig. 2). Under these circumstances all the lobules, both large and small, were enlarged and no individual lobules or groups of lobules showed any preferential enlargement.

The mitotic activity of the liver cells reached its maximum intensity on the third day [6, 9, 10, 12, 13]. The weight of the residual lobes of the liver at this time was 70-80% of the weight of the whole organ [10].

The increase in the dimensions of the hepatic lobules is thus primarily the result of an increase in the number of liver cells.

The rapid compensatory reaction of the body in this case was thus not one of formation of new structural components of the organ, i.e., lobules, but of their hypertrophy. On the 7th day after partial hepatectomy, the anatomical structure of the liver was in no way indistinguishable from the picture that was observed on the 3rd day: the hepatic lobules remained hypertrophied.

The reorganization of the structure of the liver during regeneration of the organ evidently took place mainly during the first days, since on subsequent days it was impossible for us to detect any essential difference between the dimensions of the lobules at the early and late stages of regeneration. The differences in the areas of the lobules in the experimental animals (for example, on the 14th and 20th days of regeneration), given in the table, depended on individual variations and were not statistically significant. The hepatic lobules, which doubled or trebled their size in the initial stages of regeneration, maintained their large size later on, even for 6 months (the end of our period of observation). The filling of the blood vessels had returned to normal at this time (Fig. 3).

It must be pointed out that hypertrophy of the lobules took place not only at the periphery of the organ, but also in its depths, since in the deeper sections were observed enlarged complex and simple lobules, cut transversely and longitudinally. We were never once able to discover signs of formation of new lobules, which would inevitably have been accompanied by the appearance of new efferent ducts and blood vessels.

It can be concluded from our findings that after removal of the left lateral and central lobes of the liver, hypertrophy of the lobules of the remaining portion of the liver takes place.

Regarding the possibility of formation of new lobules, both in the experiments which we carried out and in any other type of damage to the liver, in perforating wounds [1, 2] or in death of part of the parenchyma as the result of pathological conditions, there is need for special investigation.

SUMMARY

60-70% of the liver was removed by Higgins and Anderson's method in white male rats, weighing 115 to 150 g. The changes in the size of hepatic lobules in regenerating liver were studied for a period of 1 to 180 days. India ink was injected into hepatic arteries which made possible clear delineation of individual lobules. As demonstrated, the size of the lobules increases 2-3 times during the 1st — 3rd days and remains so up to 6 months months. No newly formed lobules were revealed.

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HYPOPHYSEAL INHIBITORS. THE EFFECT OF SYGETIN ON UTERINE DEVELOPMENT IN THE SEXUALLY IMMATURE MOUSE

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In our previous research [1] it was shown that in the course of physiological aging in women a period is observed which is characterized by increased activity of several divisions of the diencephalohypophyseal system.

This condition leads to an increase with advancing age of hypercholesteremia, of adiposis and of the incidence of hypertension, diabetes mellitus and certain other pathological disorders. The increased activity of the diencephalohypophyseal system is also of definite importance in the course of carcinoma of the breast, which is shown in particular by the possible therapeutic effects of estrogens, in spite of the increase which they cause in the proliferative processes in the breast. An increase in the activity of the diencephalon also lies at the basis of the development of the menopause and of the period which precedes it, of persistence of follicles developing as the result of excessive secretion of the follicle-stimulating hormone of the hypophysis [1].

These findings made it imperative to investigate the possibility of depression of the increased diencephalo-hypophyseal activity. Sex hormones – mainly the estrogens – are at the present time among the most active measures in this direction. The presence of marked peripheral effects, however, makes their clinical use much more difficult. The separation of the central effect of the estrogens and their specific estrogenic action is of great importance in this respect. Dodds [4] considers that such a separation is impossible. With the development of p-oxypropiophenone [3], however, in which the central action is retained but the estrogenic action is considerably weakened, this view must be altered.

EXPERIMENTAL METHOD AND RESULTS

In view of the above, we studied the action of sygetin, an analog of synestrol prepared by S. F. Torf and N. V. Khromov-Borisov.

This substance was put forward as a water-soluble estrogen and was tested in accordance with the usual methods of administration of the estrogens [2].

In order to determine the relationship between the central and estrogenic effects, we administered sygetin to sexually immature female white mice (weighing 7.0–9.0 g) for 3 weeks, i.e., for the period during which sexual maturation was observed to be completed in the control group. We regarded the preservation of the infantilism of the sexual organs, as judged by the weight of the uterus, as an index of the depression of the gonadotropic function of the hypophysis.

The following results were obtained. Sygetin in a dose of 100–200 γ/day, given in the form of two subcutaneous injections, totally inhibited sexual maturation (Table 1, see figure).

TABLE 1

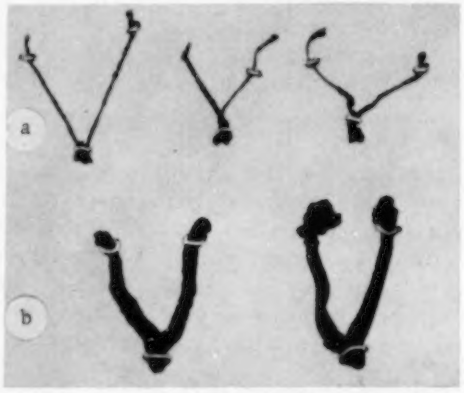
The Effect of Administration of Sygetin on Uterine Development in Mice

Serial No.	Duration of observations	Daily dose (in γ)	Weight of uterus (in mg)	
			Experiment	Control
1	From 11/23/57 to 12/13/57	20	13,6	36,0
2		20	10,1	23,0
3		20	42,2	—
4	From 2/27/58 to 3/21/58	100	9,0	85,0
5		100	10,5	24,0
6		100	5,5	88,0
7	From 6/23/58 to 7/16/58	200	8,5	60,0
8		200	9,0	78,0
9		200	8,0	—
Mean results, not counting those relating to No.3			9,3	56,2

TABLE 2

The Effect of Implantation of Tablets Containing 5 mg of Sygetin on Uterine Development in Mice

Duration of observations (in days)	Weight of uterus (in mg)	
	Experiment	Control
19	7,0	55,0
19	10,0	60,0
20	5,0	45,0
20	9,0	58,0
20	7,0	—
30	12,0	—
30	10,0	—
35	14,0	—
39	45,0	100,0
44	47,0	



Delay in the development of the uterus in mice by sygetin. a) In six-week-old mice, receiving the preparation; b) in control animals of the same age.

The action of the preparation was also shown when given by implantation of cholesterol-lanolin tablets containing 15% sygetin (5 mg), and in these conditions, moreover, restoration of the gonadotropic function was observed after complete absorption of the tablet (on the 5th-7th week)(Table 2).

Sygetin, synthesized as a water-soluble estrogen, is thus a drug in which the central action (depression of gonadotropins) takes place in doses which have no estrogenic effect.

SUMMARY

In connection with the study of the role played by the increase of diencephalohypophyseal activity with age in the development of a series of pathological processes, the author investigated the effect of sygetin on the sexual maturation of mice. It was demonstrated that sygetin is a diencephalohypophyseal inhibitor.

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MORPHOLOGY AND PATHOMORPHOLOGY

HISTOCHEMICAL INVESTIGATIONS OF THE "KUL'CHITSKII CELLS" IN THE GASTROINTESTINAL TRACT OF EXPERIMENTAL ANIMALS

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Peculiar tumors, called carcinoids, develop from the mucosa of the alimentary tract. It has been shown that carcinoids originate in the cells which were described in 1897 by N. K. Kul'chitskii [1]. He found them in the mucosa of the stomach, the duodenum and the large intestine, and showed that they secrete granules, differing in their nature from the granules of the blood cells and of the fat cells of the connective tissue. Similar cells were later described by Yu. M. Lazovskii [2] in the mucosa of the pancreatic duct, and by Detril [7] in the mucosa of the bile ducts. When examined histochemically, the cells studied by N. K. Kul'chitskii were found to have argentaffin and chromaffin properties and to give a positive diazo reaction.

Erspamer and Asero [9] isolated from the metastases of a carcinoid tumor a substance which they called enteramine, since the tumors were derived from the mucosa of the intestinal tract. These authors showed that enteramine was identical with 5-oxytryptamine, which was synthesized by Hamlin and Fischer [14].

Rapport, Green and Page [24] isolated this same substance from the serum of cattle, and called it serotonin, since it possessed a tonic action on smooth muscle.

With regard to the identification of the serotonin present in the enterochromaffin cells with 5-oxytryptamine, the investigations of Benditt and Wong [4] are of great interest; by the use of a histochemical method they showed that 5-oxytryptamine is present in these cells in a 1% concentration. Evidence in favor of the identity of the serotonin of the granules of the enterochromaffin cells with 5-oxytryptamine is given by the variations in the 5-oxytryptamine content after the administration of certain drugs, and also by the 10- to 60-fold increase in the 5-oxytryptamine content in carcinoid tumors [4, 17, 21, 23, 28, 29].

Serotonin is widely distributed in the body; about 99% of the total content is in the intestine, the blood platelets and the brain, especially in the brainstem (cited from Gennes and de Fossey [13]). It causes contraction of the smooth muscle of the internal organs and vessels; the vessels most sensitive to it are those of the kidneys [22]. Reserpine, when given to an animal, causes the disappearance of serotonin from the enterochromaffin cells for several hours, and liberates endogenous serotonin from the brain tissue [20]. The serotonin liberated by reserpine is destroyed by the enzyme monoaminooxidase [25].

Zbinden, Pletscher and Stuber [27] showed that the cells of the enterochromaffin system react differently to the administration of reserpine. In the pyloric cells, for instance, the granules disappear, whereas in the mucosal cells of the fundus of the stomach even lethal doses of reserpine do not lead to disappearance of the argentaffin granules. Evidently the function of the cells of the enterochromaffin system varies in the different parts of the body.

The results of few histochemical investigations of the "Kul'chitskii cells" have as yet been published, in either the foreign or Soviet literature. Insufficient work has been done on their distribution in the gastrointestinal

Fig. 1. "Kul'chitskii cells" in the mucous membrane of the duodenum of a guinea pig. Fontana's modification of Masson's method. Magnification: ocular 7×, objective 8×.

tract of different animals, and on the degree of packing with granules in relation to the species of animal and its physiological state.

When detecting serotoning histochemically in the cells of an animal, it must be appreciated that there is still no absolute proof that the granules of the "Kul'chitskii cells" are identical with serotonin. Morphological investigations have to be supplemented by biochemical methods of checking, by color chromatography and so on. No less than three histochemical reactions must be carried out: argentaffin, chromaffin and diazo reactions. Argentaffin cells possess the property of reducing silver on account of endogenous substances. The chromaffin reaction is a specific histochemical test for polyphenols, aminophenols and ortho- or paraphenols. The diazo reaction is used to exclude the presence of lipofuscin, for granules of the latter substance are not stained by diazonium salts nor their decomposition products.

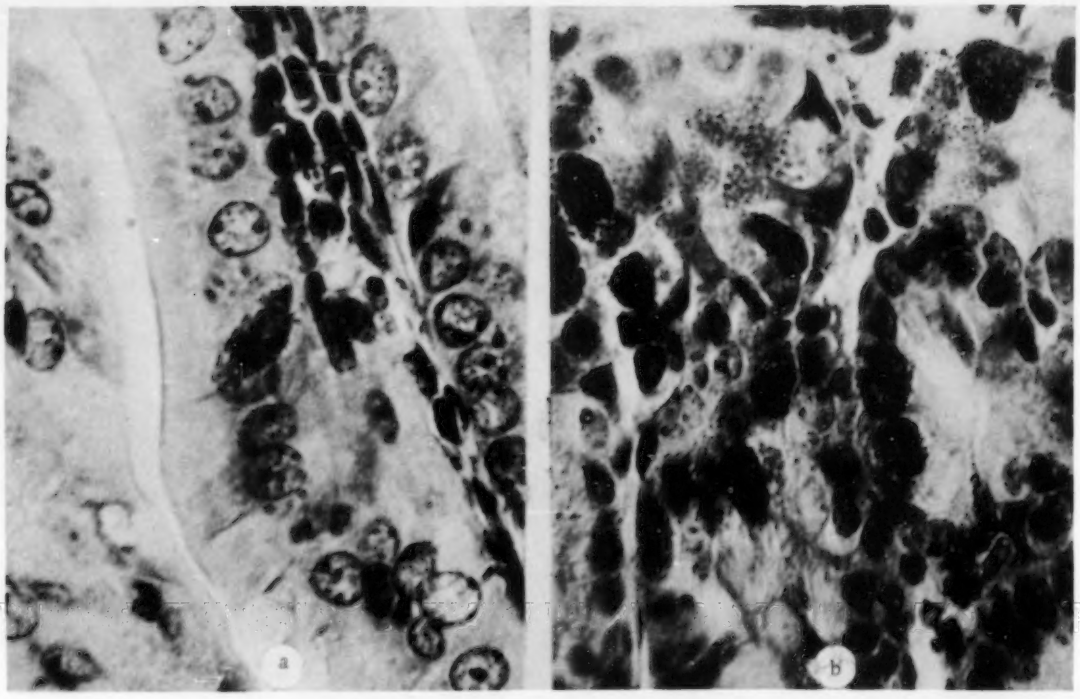

Fig. 2. "Kul'chitskii cells," situated in the crypts of the duodenal mucous membrane of a guinea pig. a) Cells, densely packed with argentaffin granules; b) a similar cell, lightly packed with granules, at the apex of a villus in the same preparation. Fontana's modification of Masson's method. Magnification: ocular 7 x. objective 40 x .

We investigated the content of "Kul'chitskii cells" in the gastrointestinal tract of different experimental animals. The aim of the present research was to examine the distribution and degree of packing of these cells with granules, throughout the gastrointestinal tract of laboratory animals in order to discover the most convenient and economical model on which to study pharmacologically active compounds possessing a reserpine-like action or the property of inhibiting monoaminooxidase (compounds of great importance in the practice of neuropsychiatry).

EXPERIMENTAL METHOD

We examined the gastrointestinal tract of monkeys, cats, rabbits, guinea pigs, white rats, mice, birds and frogs, 120 animals altogether. The animals were kept on the ordinary nursery diet, apart from 20 guinea pigs which were in a fasting condition. The specimens from the healthy monkeys were obtained from the Institute for the Study of Poliomyelitis. In order to examine the effect of different physiological conditions on the degree of packing of the "Kul'chitskii cells" with argentaffin granules, an experiment was carried out on fasting animals. Guinea pigs, weighing 330-400 g, were kept in a specially contructed cage, with a grating over the floor to prevent any possibility of the animals eating their own feces. The guinea pigs received water but no food until they died from starvation. All the animals were sacrificed by decapitation, the starving animals 2, 3, 5, 6 and 7 days from the beginning of the experiment; furthermore some of the guinea pigs died on the 8th day of starvation. The killed and dying animals were examined post mortem, and after inspection of the internal organs, samples of the wall of the stomach, duodenum, jejunum and large intestine were taken for morphological examination. For the argentaffin and diazo reactions, the material was fixed in 12% neutral formalin, and for the chromaffin reaction, fixation was for 24 hours in a mixture of 9 parts of bichromate (3.5%) and one part of neutral 40% formalin. In order to reveal argentaffin granules we used Masson's method, with Hamperl's formula and using Fontana's solution. The chromaffin reaction was carried out by Pfeiffer-Jarisch method (for a description of the methods [3]).

EXPERIMENTAL RESULTS

Histochemical investigation showed that the clearest granules were observed by the use of the argentaffin stain, and less clear by the diazo reaction; the chromaffin reaction gave diffuse staining of the protoplasm of the "Kul'chitskii cells".

In the gastrointestinal tract of the guinea pigs we found an abundance of argentaffin cells, densely packed with granules, mainly in the mucous membrane of the duodenum; considerably fewer of them were found in the mucosa of the fundus of the stomach, the jejunum and large intestine, and rather more at the outlet of the small intestine into the large; a few cells were seen in the mucosa of the hepatic and pancreatic ducts. In the thickness of the mucosa of the duodenum the "Kul'chitskii cells" were distributed unevenly: there were more of them in the crypts, fewer in the villi, and only solitary cells were found at the apex of the villi; there was a falling off also in the packing of the cells with granules as the apex of the villus was reached (Fig. 1).

In a transverse section of the intestine it could be seen that areas of the mucosa situated over lymphatic follicles were almost free from these cells. Other characteristic features were the arrangement of the argentaffin cells in the membrane between the epithelial cells, their triangular or oval shape and the absence of any contact between their protoplasm and the surface of the lumen of the intestine (Fig. 2, a, b).

The content of granules in the cells varied enormously, even in normal animals. In adults, cells were present which were packed with an enormous number of granules, so that the nucleus was invisible; in other cases its outline could be made out and, finally, there were cells in which a few hardly perceptible specks were present. In general, in healthy adult guinea pigs, enterochromaffin cells densely packed with granules were predominant. On transverse section of the duodenum, in different animals, from 450 to 1000 cells could be counted, and in other divisions of the intestine, from 0.25 to 100 cells.

In white rats the number of enterochromaffin cells was considerably smaller and the granules were small and not so numerous; they were sometimes distinct from the surface of the protoplasm in the form of strips, whereas in guinea pigs the granules were scattered over the whole surface of the protoplasm of the cell. In rabbits and mice the number of enterochromaffin cells, and the extent to which they were packed with granules, were also smaller than in the guinea pigs, but the shape of the cells and the distribution of the granules were identical. In monkeys, enterochromaffin cells were also found most often in the mucous membrane of the duodenum, but much more rarely than in guinea pigs. Their shape and distribution were similar to those described in man. We found cells, reasonably well packed with granules, in the mucous membrane of the duodenum in fowls and chicks; in the remaining divisions of the intestine, solitary cells, poor in granules, were seen, and in the wall of the stomach they were absent. In the frog the number of enterochromaffin cells in the mucosa of the alimentary tract was insignificant, and the granularity was represented by a slight accumulation of formations resembling dust.

The diazo reaction gave clear results in cases when the argentaffin granules were well represented, but by the use of the first method the granules had indistinct outlines. With the chromaffin reaction the protoplasm of the cells was dissusely stained and the outlines of the granules could not be distinguished.

In view of the difference in the content of cells of the enterochromaffin system in the animals investigated, we consider that the most suitable for experimental research are guinea pigs and chicks, for in these species it is easier to make quantitative estimations of the changes in the cell composition and in the degree to which the cells are packed with granules.

Microscopic examination of the alimentary tract of the starving guinea pigs showed the gradual diminution in the granularity of the enterochromaffin cells, although this did not completely disappear, even in the animals dying from starvation. It was also found that after intraperitoneal injection of reserpine, in a dose of 10 mg/kg, the argentaffin cells disappeared in normal animals but persisted in the starving animals. After the same dose of reserpine, given by mouth, the granules did not disappear. It is evident that in the starving guinea pigs, decomposition of the reserpine took place after its parenteral injection.

SUMMARY

The authors conducted a histochemical study of the distribution of "Kul'chitskii cells" and their saturation with the granules in various laboratory animals. The argentaffin and chromaffin reactions were determined

with a parallel diazo reaction. An uneven distribution of "Kul'chitskii cells" along the intestine was revealed. The highest density of the cells and the greatest granule saturation were observed in the duodenal mucous membrane of cats, rabbits, guinea pigs, white rats, white mice, monkeys and chicks. The content of argentaffin granules is slightly decreased in the cellular protoplasma of fasting guinea pigs, but even in animals dead of hunger they do not disappear completely. After intraperitoneal injection of reserpine, argentaffin granules vanish in normal animals, but not in fasting ones.

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HISTOGENESIS OF THE PLASMA CELLS IN EXPERIMENTAL Q-FEVER IN GUINEA PIGS

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In the last ten years many papers have appeared indicating the synthesis of antibodies by plasma cells [9, 10, 11]. This has acted as a spur to intensify the study of the origins of the plasma cells [5, 11, 13]. An outstanding monograph on this subject has been published by Burnet and Fenner [8].

Research workers express contradictory opinions on the origin of the plasma cells. Certain authors state that these cells are derived from cells of the reticular tissue [2, 6, 7, 11, 16, 17]. Others [3, 4, 12] consider that plasma cells originate from lymphocytes. Arinkin [1] distinguishes four types of plasma cells depending on their origin: erythroblastic, lymphocytic, myeloblastic and reticuloendothelial. Unna [19] and Pappenheim [15] emphasize that plasma cells are derived from histiocytes. Schridde [18] and Naegeli [14] consider that a myeloblastic genesis of plasma cells is a possibility.

The purpose of the present work was to examine the histogenesis of the plasma cells during experimental Q-fever in guinea pigs.

EXPERIMENTAL METHOD

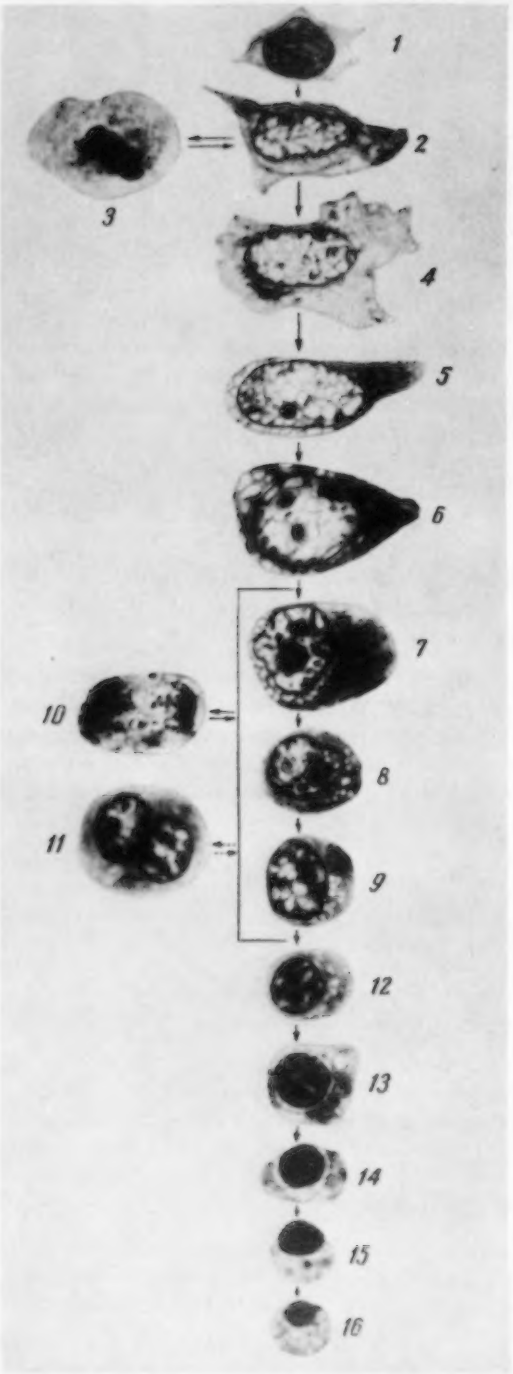
The investigation was carried out on 40 animals inoculated subsutaneously, in the left inguinal region, with a rickettsial suspension (Italo-Greek strain) in a dose of 10,000 infecting doses for guinea pigs.

Material for histological examination was obtained by sacrificing two animals on each of the following days after inoculation: 2nd, 4th, 6th, 8th, 10th, 12th, 14th, 16th, 20th, 25th, 30th, 40th, 50th, 60th and 90th. The spleen, the regional lymphatic glands and the common mesenteric lymphatic gland were fixed in Zenker formol and embedded in paraffin wax. Sections were cut to a thickness of 5 μ and stained with azure II-eosin.

EXPERIMENTAL RESULTS

In the spleen and lymphatic glands a distinctive reaction was observed, consisting of a rapid increase in the number of plasma cells in the red pulp of the spleen and in the fleshy cords of the medullary zone of the lymphatic glands, against a background of marked swelling and hyperplasia of the cells of the reticular tissue and the endothelium of the sinuses.

On the 4th-6th day after inoculation, in the spleen and lymphatic glands there appeared accumulations of cells, characterized by basophilia of their cytoplasm and by a large nucleus, cells poor in chromatin and with one or two large nucleoli. Later (8th-12th day), among these cells appeared an increasing number of typical plasma cells and plasma cells in various intermediate stages of maturation. Between the 12th and 30th days from the moment of inoculation, a dynamic equilibrium was observed between the basophilic cells on the one hand and immature and mature plasma cells on the other. Finally, there was a decrease in the number of basophilic cells with, at the same time, an increase in the typical plasma cells, especially noticeable between 40 and 50 days after the moment of inoculation.



Genesis of plasma cells during experimental Q-fever in guinea pigs. 1) Normal reticular cell; 2) changed reticular cell after inoculation of animal; 3) mitosis in a changed reticular cell; 4, 5) transitional cells; 6) plasmoblast; 7-11) immature plasma cells; 12) mature plasma cell; 13-16) degenerating plasma cells. Stained with azure II-eosin. Assembled from microphotographs. Magnification: ocular 20x, objective 90x.

From the sequence of this reaction it may be supposed that the plasma cells were the differentiated forms of the basophilic cells described above. The question naturally arises of the origin of these basophilic cells. In the early stages it could clearly be seen that the reticular cells increased in size, attaining 12-14 μ in diameter (normally about 8 μ). In shape they were polygonal, often having long processes, and their cytoplasm had a fine cellular structure. The nucleus was oblong in shape, comparatively poor in chromatin, sometimes vesiculiform and with one or two nucleoli, the latter being relatively small in size. A proportion of such cells were in a state of karyokinetic division (see figure, 2 and 3).

The process of differentiation of the plasma cells begins, in the author's opinion, with the appearance of a basophilic cytoplasm in individual reticular cells, mainly around the nucleus (see figure, 4). With increasing basophilia of the cytoplasm, the reticular cell begins to lose its syncitial connection with the other cells and to become round in shape. The nucleus also becomes round in shape (see figure, 5) and under these circumstances there is an increase in the dimensions of the nucleolus. Cells which have lost their syncitial connections and become round in shape, resemble lymphoblast with a mean diameter of 13-14 μ (see figure, 6).

In this way are formed the accumulations of basophilic cells which can be seen at different periods (2, 4, 6 days). This is followed by the process of maturation of the plasma cells (see figure, 7-11). It is characterized by a decrease in the size of the cells of the lymphoblast type, of their nucleus and nucleoli, by an increase in the density of chromatin, by increased basophilia of the cytoplasm and by the appearance of small vacuoles in these cells, around the nuclei. As a rule the nucleus is situated eccentrically. Plasma cells in the stage of maturation are capable of mitotic and, evidently, amitotic division (see figure, 10-11).

The diameter of the cells diminishes from 14 to 8 μ during maturation. The degree of maturation of the plasma cells may be judged not only by dimensions of the cell itself, but by those of the nucleolus, in addition to the signs already mentioned. In the adult plasma cell, the nucleolus is insignificant in size and is often ill-defined in the comparatively dense chromatin of the nucleus (see figure, 12).

Starting on the 16th day, and at later periods, an increasing number of plasma cells with pyknotic nuclei were seen. These were evidently degenerating plasma cells (see figure, 13-16). The process of degeneration of the plasma cells began with an increase

in the density of the nuclear chromatin, with a gradual loss of its visible structure and a decrease in its size. The cytoplasm lost the power of staining intensively.

The whole cell was reduced in size. Some of these cells were engulfed by macrophages, others evidently underwent lysis.

From the morphological findings it may be concluded that plasma cells are derived from the reticular cells of the spleen and lymphatic glands. The individual cells indicated in the figure require a precise nomenclature Fagraeus [11] conventionally divides all the cells into three groups: transitional cells, immature plasma cells and adult plasma cells. This terminology is in agreement with the course of development of these cells, but requires clarification.

The cells which are called transitional by Fagraeus are those which are lying freely, i.e., have lost their syncitial connections. The results obtained show that the name "transitional cells" is best applied to reticular cells which are changed as the result of an increase in the basophilia of the cytoplasm, which are in process of losing their syncitial connections, but have not lost them completely (see figure, 4-5).

When the transitional cell has finally lost its syncitial connection it becomes free, and somewhat resembles a lymphoblast, differing from it by its more extensive and basophilic cytoplasm and its situation. These cells should be called plasmoblasts (see figure, 6). It was these cells that Fagraeus, judging from his description, called transitional cells. By means of the conversions described above, from plasmoblasts were obtained first, immature and then later, mature plasma cells (see figure, 7-12).

Nothing is said about the subsequent fate of the plasma cells in Fagraeus's paper. He merely observes that the decrease in the numbers of mature and immature forms takes place simultaneously, although mature form disappear more slowly than the immature. Fagraeus gives no morphological analysis of this process.

Our findings show that the mature plasma cells subsequently degenerate. Arinkin [1], in puncture material from lymphatic glands, saw basophilic formations which were round or oval in shape, the size of a normocyte or sometimes larger. He considers that they were remnants of plasma cells. Arinkin was unable to follow their subsequent fate.

In developing our point of view on the genesis of plasma cells, we cannot at present, without suitable verification, deny that there are other possible sources from which plasma cells may originate when different factors act on the body.

SUMMARY

The paper deals with the origin of plasma cells in guinea pigs during experimental Q-fever. The author succeeded in demonstrating that plasma cells originate from the reticular cells of the spleen and lymph nodes. The development of plasma cells into reticular ones is distinguished by the following phases: 1) transitional cells, 2) plasmoblasts, 3) immature plasma cells, 4) mature plasma cells and 5) degenerating plasma cells.

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METHODS

A PHOTOGRAPHIC METHOD OF RECORDING THE ARTERIAL BLOOD PRESSURE

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We have devised an apparatus for recording the arterial blood pressure photographically, consisting essentially of a mercury manometer, a photographic lens and a photokymograph.

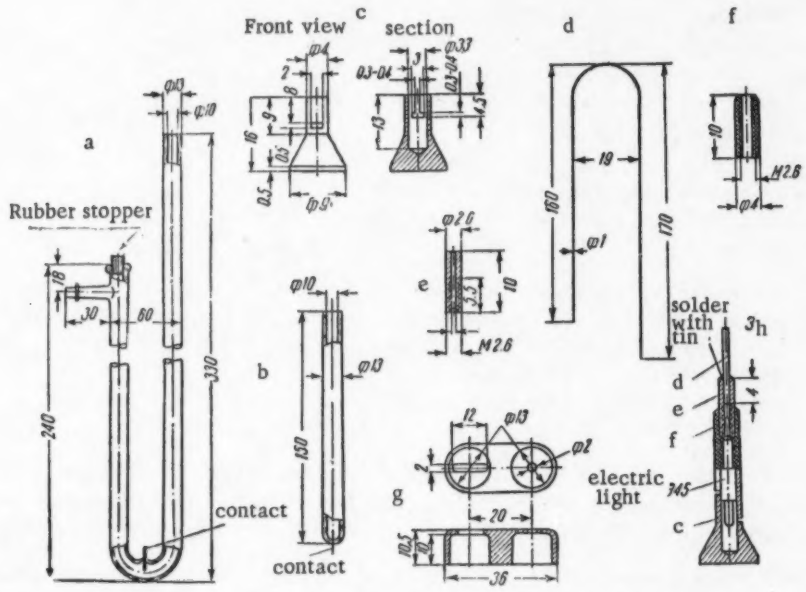

Fig. 1. Outlines of the main details of the manometer. a) V-shaped glass tube with soldered-in contact; b) straight glass tube with soldered-in contact; c) stainless steel float; d) steel wire contact; e) brass connector; f) ebonite bush; g) brass adjusting cap; h) outline of assembled details c, d, e, f, and E-45 electric lamp (where it is impossible to solder the contacts in parts a and b, they may be introduced into the mercury through a vertical orifice in the left tube of part a, closed with a rubber stopper, and from the cap g in part b).

The scheme of this apparatus is shown in Fig. 2. In the tube of a mercury manometer 1 is placed a miniature electric light 2, mounted in a float 3, in the wall of which is a small opening for the passage of light. The

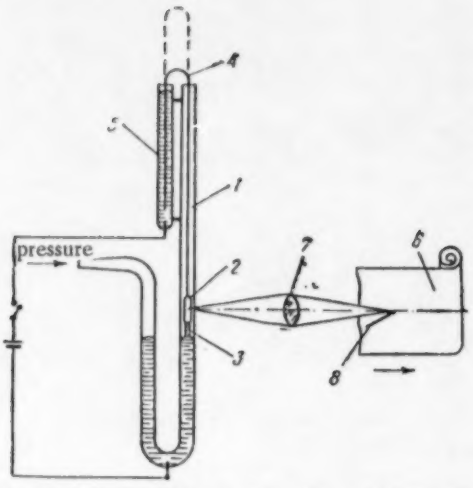

Fig. 2. Scheme of the manometer. 1) Manometer 2) electric light E-45, as fitted to endoscopic apparatus; 3) stainless steel float; 4) wire contact; 5) tube with mercury contact; 6) photographic paper; 7) photographic lens with focal length 135 mm; 8) pressure tracing curve.

lamp is connected to the central contact by means of a steel wire 4, bent to form a loop, the short end of which is placed in a tube 5, filled with mercury.

In the lower, closed ends of the manometer 1 and the tube 5 are soldered electrodes, in contact with the mercury, to which are applied an emf of 3 volts (for example two dry cells). The current to the lamp passes through the mercury contacts of the tubes 1 and 5, and through the float 3 and the wire 4 respectively.

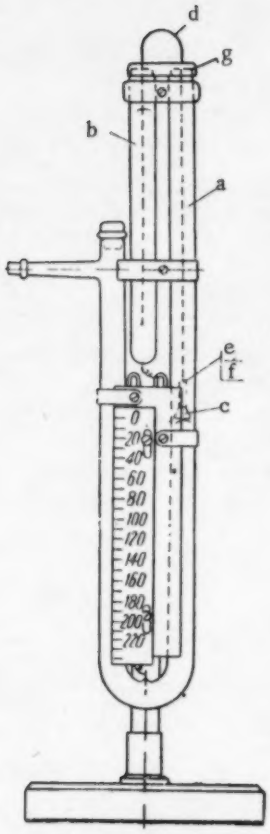

Fig. 3. General appearance of the manometer; legend as in Fig. 1.

When the arterial pressure rises, and with it the level of the mercury in the manometer, the float 3, together with the lamp 2, moves upwards along the tube 1. The displacement of the lamp is recorded on the moving photographic paper 6 of the photokymograph by means of the lens 7, in the form of the curve 8.

The lens 7 is immovably situated between the manometer and the photokymograph, at a suitable distance to allow the formation of a sharp image of the filament of the lamp on the photographic paper. The dimensions of the image are restricted by the aperture in the float 3.

This method of recording enables the arterial pressure to be traced on ordinary photographic paper simultaneously from several manometers, using one lens.

By this method it is possible to record changes in the arterial pressure both during an acute experiment and when a sphymometric method of recording is used.

The scale of the pressure tracings can be varied at will, depending on the conditions of the experiment and the width of the photographic paper. The quality of the curves permits pressure changes to be distinguished with an accuracy of 1-2 mm Hg.

Construction of the manometer is simple, and may be carried out in an ordinary workshop in accordance with our diagrams (Figs. 1 and 3).

SUMMARY

The paper presents a description of a photographic device for recording the arterial blood pressure. Its main feature is a mercury manometer equipped with a miniature incandescent lamp fixed on a float in the manometer tube.

Low voltage electric current is supplied to the lamp through mercury contacts. The float with the lamp follows all the fluctuations in the mercury level of the manometer.

The image of the lamp filament is registered on a photokymograph , in the shape of a curve having a variable amplitude, with the aid of an ordinary photographic lens.

The article includes drawings of the main parts.

A METHOD OF MEASURING THE MINUTE VOLUME OF THE HEART

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(Received June 6, 1958. Presented by Active Member AMN SSSR V. V. Zakusov)

The methods most widely used for determining the minute volume of the heart are Fick's direct method and a method based on measurement of the rate of dilution of a dye [1, 3, 5]. These methods enable measurement of the minute volume during both acute and chronic experiments; they are, however, very complicated and laborious. There are simpler methods which are based on the direct measurement of the volume of blood flowing through the heart. For this purpose a flowmeter is used, by means of which the volume rate of the blood flow is measured in the venae cavae [4], the aorta [2, 7] or the pulmonary artery [6].

In the present communication we describe a method of measurement of the minute volume of the heart which requires no special apparatus. The method involves measurements of the volume rate of the blood flow in the pulmonary artery. The low level of the blood pressure, and the possibility of measuring the whole quantity of blood flowing through the heart, including the blood from the coronary vessels, makes the pulmonary artery the most suitable place for measurement of the minute volume.

A suitable animal in which to measure the minute volume is a cat weighing 3-4 kg. Under nembutal anesthesia, and with artificial respiration, thoracotomy is performed. To prevent clotting of the blood, heparin (1000-1500 units/kg) is injected into the animals. The pulmonary artery is separated from the surrounding tissues and two cannulas are inserted through an incision in its wall: one into the central, the other into the peripheral end of the artery. The pulmonary artery must not be clamped for longer than 30-40 seconds during insertion of the cannulas if fibrillation of the heart is to be avoided.

The cannulae to be inserted into the pulmonary artery are made of polyvinyl chloride tubing. The narrowest point of the cannula must be not less than 3 mm in diameter; this is necessary to reduce the resistance to the flow of blood. Because the length of the pulmonary artery in the cat does not exceed 1.5-2.0 cm, the ends of the cannulae designed for insertion into the artery are made short — 4-5 mm. After the cannulae have been inserted into the pulmonary artery, the blood at once begins to flow into a special apparatus designed to measure periodically the volume rate of the blood flow (Fig. 1).

As will be seen in Fig. 1, the blood entering the cannula inserted into the central end of the artery passes along the tubes 4 and 1 and is returned into the peripheral part of the pulmonary artery. The clamp which is situated on tubes 1, 2 and 3, under these circumstances is in position I.

When the clamp is moved to position II, blood cannot enter the tube 1, but is directed along tube 3 into a rubber balloon, situated in the reservoir C. The balloon is distended and displaces water from the reservoir C into the glaa tube B. In this way the float, to which is attached a pen, is raised, and a sloping line is traced on the moving drum of the kymograph. The minute volume is determined from the angle which this makes with the base line.

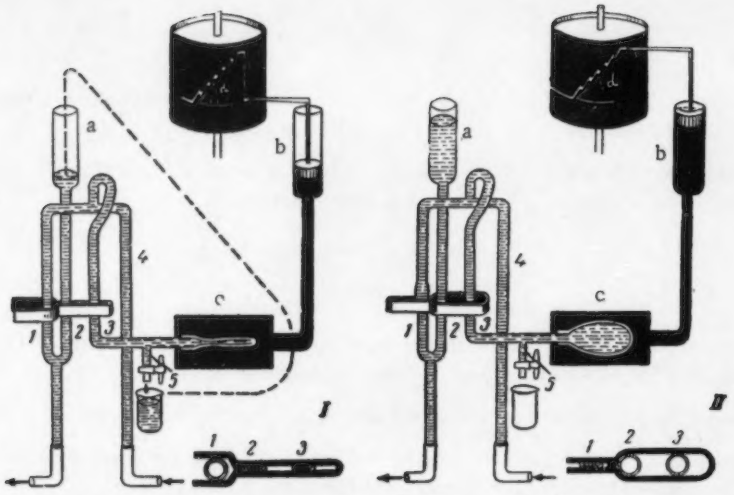

Fig. 1. Scheme of the method of determining the minute volume. For description see text.

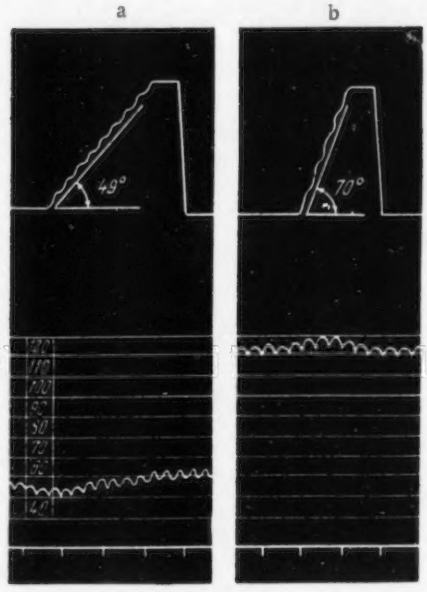

Fig. 2. Measurement of the minute volume before (a) and after (b) injection of adrenalin in a dose of 50 γ / kg.

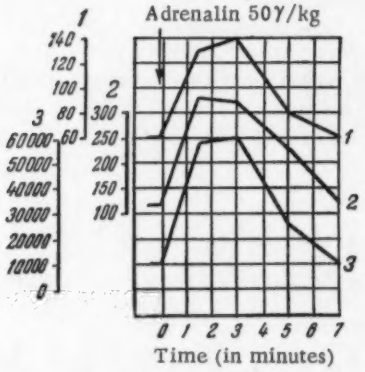

Fig. 3. The effect of adrenalin on the minute volume and the work of the heart. 1) Arterial pressure in mm Hg; 2) minute volume in ml/ min; 3) work of the heart in g/ cm/ min.

In order to compensate for the cessation of the blood flow to the lungs, at the same time as blood enters the reservoir C, blood from the reservoir A is directed along the tube 2 into the peripheral end of the pulmonary artery. The reservoir A, like the tube B, is at a height above the level of the heart corresponding to the mean value of the pressure in the pulmonary artery of the cat (20-25 cm).

The length of time taken to determine the volume velocity of the blood flow in the pulmonary artery is 1.5-3.0 seconds on each occasion. During this time 4-6 ml of blood enters the recervoir C. At the end of this time, the clamp is moved back again to position I. The blood filling the rubber balloon flows out through the tube 5 and is taken into recervoir A.

The minute volume is calculated from the formula

$$M = (AVt)\,\mathrm{tgd},$$

where A is the scale of magnification of the elevation of the float and pen in ml/cm; V is the velocity of movement of the drum of the kymograph in cm/sec; t — time in seconds.

In our experiments the values A and V were constant (A = 2 ml/cm, V = 1 cm/sec), and during measurement of the minute volume, t was also constant and equal to 60 seconds, hence

$$M = C\,\mathrm{tgd},$$

$$C = AVt = 2 \cdot 1 \cdot 60 = 120.$$

The determination of the minute volume thus resolves itself into finding the tangent of the angle formed by the sloping line which is traced when the float and pen are raised (Fig. 2).

In our experiments the minute volume was 30-50 ml/kg body weight of the cat.

In Fig. 3 are shown curves of the minute volume and the work of the left heart, obtained by means of the method described. The work of the left heart is calculated from the formula

$$A = 13.6\,MP$$

where A is the work of the heart in g/cm/min; M is the minute volume in ml/min and P is the systolic arterial pressure in cm Hg.

SUMMARY

A method of measuring the minute heart volume requiring no special instruments is presented. The minute volume is determined by direct measurement of the blood flow volume velocity in the pulmonary artery. A special device for the periodical determination of the blood flow volume velocity is described.

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A MODIFICATION OF THE VISCOSIMETRIC METHOD OF MICROESTIMATION OF THE HYALURONIDASE ACTIVITY OF BIOLOGICAL FLUIDS

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Laboratory and clinical studies of the hyaluronidase activity of biological fluids have often been limited by the lack of a simple and reliable micromethod for estimation of this enzyme. The existing macromethods require 1.5-2.0 ml of test fluid for the analysis. The micromethods so far described are either complicated or are designed for special purposes.

In the suggested micromodification of the viscosimetric method [7], the principle of the capillary type of viscosimeter [5] is used (see figure). The apparatus is placed in a thermostatically controlled water bath, for which purpose an aquarium or a glass accumulator case with a heating system may be used.

The investigations are carried out at a temperature of 34°. Into the funnel of a viscosimeter is poured 0.05 ml of the test solution, 0.05 ml of citrate buffer at pH = 4.6 and 0.2 ml of a 0.2 % solution of hyaluronic acid. The total volume of the mixture corresponds to the capacity of the working part of the viscosimeter — about 0.3 ml.

The enzymic activity of the test sample is judged by its ability to lower the viscosity of the original solution of hyaluronic acid in the course of 20 minutes. At the begining and end of the investigation, the time of flow of the mixture between the upper mark and the junction with the capillary tube is measured with a seconds counter with an accuracy of 0.2 seconds.

The enzymic activity of the test solution is calculated as the decrease in the viscosity of the substrate in percent of the initial value. The conventional unit of activity is a decrease of 1% in the viscosity.

The calculation is made as follows. The time of flow of the mixture of test solution, buffer and hyaluronate at the begining of the experiment is designated t_1 and the time of flow after incubation for 20 minutes t_2. The difference $t_1 - t_2$ then gives the quantity of substrate losing its viscosity during the investigation.

The difference $t_1 - t_0$ will evidently give the initial value of the viscosity of the substrate, where t_0 is the time of flow of the test fluid and buffer without hyaluronic acid. In this series of investigations, t_0 is a practically constant value, and may be determined once only for each viscosimeter.

It is necessary to dwell in rather greater detail on this value t_0 which, in our opinion, must be affected also by products of total depolymerization of hyaluronic acid formed at the end of the investigation, which are not taken into consideration when the viscosimeter is used in the ordinary manner. We introduce the appropriate correction in such a way that, when determining t_0, a further equal volume of totally depolymerized 0.2% hyaluronic acid is added instead of distilled water to the buffer and the test fluid.

In accordance with what has been stated, the enzymic activity of the test fluid in conventional units is calculated according to the formula:

$$A = \frac{t_1 - t_2}{t_1 - t_0} \cdot 100.$$

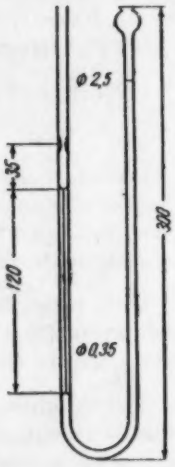
When the results of the viscosimetric determination are being evaluated, it is imperative to bear in mind that the lowering of the viscosity of the substrate is not strictly specific for the action of hyaluronidase, but may be due also to other nonenzymic factors. In parallel with the experimental test, a control was therefore set up with the test solution which had been preliminarily heated on a boiling water bath for 30 minutes to inactive the enzyme. Subtraction of the results of the control test from the value obtained for the decrease in viscosity in the experimental test gives the true activity of the enzyme.

The nonenzymic depolymerization of hyaluronic acid in the control test may be so considerable that the correction equals or even exceeds the true enzymic effect. Under these circumstances the analysis as a whole becomes insufficiently reliable.

On this basis a number of authors conclude that the mucolytic activity of the urine, determined by a viscosimetric method, is due not to hyaluronidase but to nonspecific thermostable substances [3, 4]. If the comments of these authors are justified, then doubts may be expressed about the results obtained in A. G. Ginetsinskii's laboratory from investigation of the hyaluronidase in the urine providing a basis for the expression of definite views on the mechanism of action of the antidiuretic hormone [1, 2].

A special investigation of this problem showed the following. Many samples of urine, after boiling, actually gave rise to a considerable lowering of the viscosity of the hyaluronic acid. The "mucolytic activity" of the control, boiled samples of urine, however, is not determined by their content of unknown substances of other than enzymic nature, but by the properties of the substrate itself.

Since no sufficiently purified standard preparation of hyaluronic acid manufactured commercially is yet available, each research worker usually obtains it independently. The method of McClean and Hale [6] is very widely used for this purpose, consisting of drying human umbilical cords in acetone, shredding them and extracting the hyaluronic acid with water, precipitating it with alcohol, and then drying the precipitate in ether and over $CaCl_2$. The method is very simple and gives a considerable yield of highly polymerized product, although insufficiently purified.



Scheme of the microviscosimeter (the dimensions are given in mm).

In recent years papers have appeared describing methods of complete purification of hyaluronic acid. In the usual laboratory practice, however, it is difficult to apply these methods of preparation of the highly purified product because of their complexity. Hyaluronic acid, isolated by the method of McClean and Hale, fully satisfies the requirements of the viscosimetric method, although it does have one essential defect. In some cases the hyaluronic acid undergoes spontaneous depolymerization, even after the addition of the distilled water and buffer. It this factor is not taken into consideration, the erroneous conslusion may be reached that substances of a nonenzyme nature are present in the test samples, depolymerizing the substrate, as took place in the researches of E. P. Stepanyan and N. I. Gorbarenko [3] and E. I. Chazova [4].

The cause of spontaneous depolimerization very probably is the extraction of proteins of an enzyme nature from the umbilical cords. In any case this essential defect can be overcome by a simple method. For this purpose it is sufficient, before the experiment, to warm the hyaluronic acid solution on a boiling water bath for aproximately 20 minutes (the time is chosen empirically), in order to destroy the enzymes contained in the substrate itself.

In the table are given the results of a comparative determination of the depolymerizing effect shown by various fluids, when noninactivated solutions of hyaluronic acid and solutions inactivated by boiling were used as substrate.

The Effect of Heating on the Spontaneous Depolymerization of Hyaluronic Acid

Experiment No.	Test fluid	Activity, in conventional units					
		substrate					
		noninactivated hyaluronic acid			hyaluronic acid, inactivated by boiling		
		experiment	control	activity	experiment	control	activity
1	Distilled water	12	—	12	0	—	0
2	" " "	14	—	14	1	—	1
3	Rat's urine	13	16	3	1	1	0
4	" "	25	10	15	17	2	15
5	" "	37	14	23	23	2	21

The spontaneous depolymerization of this particular hyaluronic acid amounts to about 14 units, as can be seen from the experiment with distilled water. Roughly the same value is given by the control, using noninactivated acid, during analysis of the enzymic activity of the urine. On treatment of the substrate by boiling the spontaneous depolymerization ceases and the control sample becomes stable. At the same time it must be remembered that the correction introduced into the unstable control nevertheless is adequate. In experiment No. 3, for instance, the urine shows no activity, but in experiment No. 4 it shows the same activity when tested with both substrates.

After inactivation of the substrate and the prevention of spontaneous depolymerization of hyaluronic acid, the viscosimetric method and its modifications are thus simple and reliable methods of investigation of the hyaluronidase activity of very small quantities of biological fluids. The results obtained by this method for the hyaluronidase activity of urine are perfectly reliable.

SUMMARY

A viscosimeter of capillary type was employed for the microdetermination of hyaluronidase in biological fluids. For analysis samples of 0.05 ml of the solution under study are taken.

The inconstancy of results obtained by viscosimetric method may be, in a great measure, due to the presence of spontaneous hyaluronic acid depolymerization. This defect is removed by heating the substrate. The above data prove the thermolabile enzymic nature of the mucolytic factor revealed in the urine.

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* See English translation.

** Original Russian pagination. See C.B. Translation.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for Experimental Morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

*BRITISH-AMERICAN TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
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Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
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Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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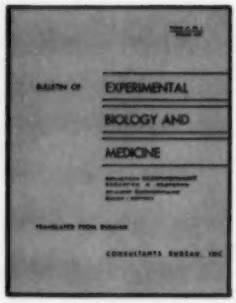

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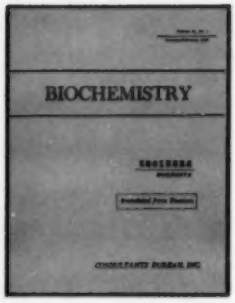

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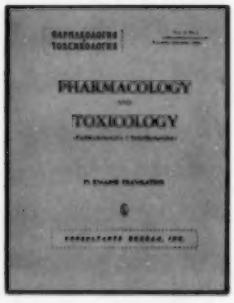

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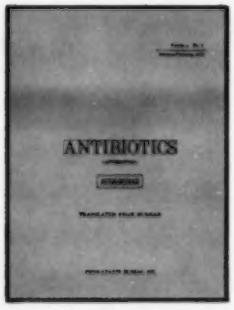

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